Clin. exp. Immunol. (1978) 33, 347–356.

Effects of human alpha-foetoprotein on human B and T lymphocyte proliferation *in vitro*

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(*Received* 21 *February* 1978)

SUMMARY

Purified human alpha-foetoprotein (AFP) isolated from extracts of foetal and hepatoma tissues, and from cord serum was evaluated as to its suppressive effects on *in vitro* lymphocyte responses to stimuli which selectively trigger human B or T cells. The effects of equivalent concentrations of individual AFP preparations were compared on lymphocyte cultures stimulated with the human B cell mitogen *Staphylococcus aureus* strain Cowan I organisms, with the T cell mitogen phytohaemagglutinin (PHA), and with irradiated allogeneic lymphocytes in the one-way mixed lymphocyte reaction (MLR). PHA responses were significantly inhibited by most purified preparations of AFP in a dose-dependent manner, within the concentration range of 300 to 18 μg/ml. However, individual foetal-derived AFP preparations did vary in suppressive potency on PHA responses, and attempts to reactivate an inactive AFP were unsuccessful. In parallel cultures the mitogenic response to protein A expressing *Staph. aureus* bacteria was normal or even slightly enhanced by AFP. The one-way MLR was effectively suppressed at higher concentrations of AFP (300–600 μg/ml) than were required for inhibition of PHA responses. The inhibitory effect of AFP on PHA-induced lymphocyte proliferation was not altered by increases in the mitogen dose. No evidence was found that AFP merely inhibits PHA responses by direct interference with mitogen or by competition for cell surface receptors with the mitogen. The results reported here indicate that human AFP effectively suppresses certain T cell-mediated reactions, but not B cell responses *in vitro*, and these are in line with previously reported findings in the murine AFP system.

INTRODUCTION

Alpha-foetoprotein (AFP) is a major protein component of human foetal serum which gradually diminishes in concentration post-natally to the low ng amounts found in adult serum (Ruoslahti & Seppälä, 1971). Elevations in serum AFP levels recur in adult females as a normal physiological event during pregnancy (Seppälä & Ruoslahti, 1972) and in patients with certain malignant (Abelev, 1971) and non-malignant (Adinolfi, Adinolfi & Lessof, 1975) diseases signifying the abnormal re-expression of an embryonic protein (Alexander, 1972). While the pathological distribution and the potential diagnostic importance of AFP is widely appreciated (Abelev, 1974), precise information concerning its various possible biological functions is largely lacking.

Recent studies in the murine system have shown that mouse AFP may have immunosuppressive properties *in vitro* on allogeneic or mitogen-induced lymphocyte transformation (Murgita & Tomasi, 1975a), as well as on primary and secondary antibody responses (Murgita & Tomasi, 1975b). Human AFP may also display an inhibitory effect on the mitogenic transformation of human lymphocytes (Yachnin & Lester, 1976; Auer & Kress, 1977; Gupta & Good, 1977; Murgita *et al.*, 1977a). The *in vitro* effects of AFP in the murine system are restricted with regard to the lymphoid target cell types

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0099-9104/78/0800-0347$02.00

that are affected. Thus, mouse AFP will effectively suppress antibody responses to T cell-dependent antigens, as well as mitogen and alloantigen stimulated DNA synthesis of purified T lymphocytes, but it fails to inhibit T cell-independent specific or polyclonal antibody synthesis (Murgita & Wigzell, 1976; Peck, Murgita & Wigzell, 1978). One mode of immunoregulatory action by murine AFP is probably through the induction of suppressor T cells which effectively interfere *in vitro* with antibody responses to T cell-dependent, but not T cell-independent, antigens (Murgita *et al.*, 1977b). These findings indicate that AFP of murine origin is capable of exerting selective inhibitory action on certain T cell functions *in vitro*.

The present investigation was undertaken to determine whether human AFP might also exert selective inhibitory effects on mitogen and on alloantigen stimulated proliferation of human B or T cells *in vitro*. The findings indicate that both hepatoma and foetal-derived AFP can inhibit lymphocyte transformation by the T cell mitogen PHA. Low concentrations of AFP required to suppress PHA responses strongly fail to inhibit mixed lymphocyte reactivity (MLR), or the mitogenic response to protein A expressing *Staphylococcus aureus* bacteria (strain Cowan I), a known human peripheral B lymphocyte mitogen (Forsgren, Svedjelund & Wigzell, 1976). Higher concentrations of AFP in the range of 300–600 μg/ml were required to significantly inhibit the MLR suggesting that the T cell population(s) proliferating against alloantigens in the mixed cultures are less sensitive to AFP-induced suppression than are PHA responsive T cells.

MATERIALS AND METHODS

Isolation of human alpha-foetoprotein (AFP). The purified AFP preparations used in this study were isolated from extracts of foetal and hepatoma tissues or from cord serum by passage over anti-AFP Sepharose immunoadsorbant columns as described in detail elsewhere (Ruoslahti, 1976). After elution from immunoadsorbants with 8 M urea, or glycine-HCl pH 3·0, the remaining small amounts of contaminating serum proteins in the adsorbant purified AFP preparations were removed by passage over a Sepharose adsorbant to which anti-normal human serum antibodies had been coupled. The preparations were fractionated by gel filtration on Sephadex G-200 and AcA 34 Ultrogel (LKB Produkter, Bromma, Sweden) columns to remove aggregated material. The human albumin preparation used throughout most of this study was isolated on the same columns employed for the purification of AFP. This purified foetal-derived albumin therefore served both as a protein control, and as a check for possible extraction of unrelated inhibitory material from the passage of AFP preparations through the several columns. In some experiments human adult serum albumin obtained from the Finnish Red Cross Transfusion Service was used. AFP and albumin obtained as described above fulfilled the strict criteria for protein purity. Thus, all preparations revealed only a single band of protein on analytical non-SDS polyacrylamide gel electrophoresis, run in alkaline conditions. In addition, the isolated proteins developed specific immunoprecipitin bands in Ouchterlony gel diffusion and immunoelectrophoresis tests when reacted against the relevant antisera (rabbit anti-human AFP or albumin), but showed no cross contamination with other serum proteins. Protein concentrations was determined by the dry weight of lyophilized material, or by radioimmunoassay according to the method of Johansson, Kjessler & Sherman, 1976.

Lymphocyte cultures. Human peripheral blood lymphocytes were separated from 50 ml quantities of heparinized blood from normal donors by centrifugation on Ficoll–Isopaque gradients. Separated lymphocytes were suspended to 2×10^6/ml in RPMI-1640 medium supplemented with antibiotics, 4×10^{-5} M 2-mercaptoethanol, and 10% pooled human AB positive serum, 5% foetal calf serum (Flow Laboratories Svenska AB, Solna, Sweden), or supplemented medium without serum. 50 μl aliquots of lymphocyte preparations containing 1×10^5 viable cells were added to triplicate wells of round-bottom microtitre plates (Falcon Plastics, Oxnard, California). AFP or albumin was either added in 50 μl volumes at the initiation of cultures or 1–2 hr prior to the addition of mitogens, in concentrations ranging from 18–600 μg/ml. 50 μl of *Staphylococcus aureus* bacteria (strain Cowan I, with a high content of protein A) containing 5×10^7 or 5×10^8 formalin-killed organisms, were finally added to the cell cultures making a total reaction volume of 150 μl. For MLR tests, 2×10^5 responding lymphocytes were cultured in serum-free conditions with 2×10^5 irradiated (2000R) allogeneic stimulator cells. Cell cultures were incubated at 37°C in a humidified 5% CO_2-air atmosphere. Mitogen responses were terminated on days 2 and 3, while MLRs were terminated on days 4, 5 and 6. 1·0 μCi of tritiated thymidine (^{3}H-TdR; specific activity 45Ci/mmol., Amersham, England) was added for the last 4 hr of culture. The cells were harvested using a multiple-sample harvestor (Skatron, Flow Laboratories, Oslo, Norway), and the total ^{3}H-TdR incorporation was measured by liquid scintillation procedures.

Mitogen binding studies. Human AFP was tested for a reaction with purified leucoagglutinin (LA) by measuring the binding of ^{125}I-labelled AFP to LA insolubilized on Sepharose 4B (LA-Sepharose, Pharmacia Fine Chemicals AB, Uppsala, Sweden). LA-Sepharose, or Sepharose particles alone (25 μl) were incubated for 20 hr at room temperature with 25–30,000 cpm of radiolabelled AFP or carcinoembryonic antigen (CEA), in 1 ml PBS containing 0.05% Tween 20. The amount of radioactivity bound to the particles was determined after washing three times with 2 ml PBS-Tween.

RESULTS

Comparative effects of human AFP on B and T lymphocyte transformation

Initial experiments were designed to examine the suppressive effects of human AFP on normal human lymphocyte responses to stimuli which selectively trigger B or T cells. Table 1 shows the comparative dose-response effects of AFP on lymphocyte proliferation induced by *S. aureus*, PHA and allogeneic cells in the one-way MLR. As can be seen the mitogenic response to *S. aureus* was not inhibited, but was usually somewhat enhanced by AFP at the concentrations tested. PHA responses were inhibited in a dose-dependent manner by AFP in amounts ranging from 300 to 18 μg/ml. The MLR was clearly less sensitive to suppression, with significant inhibition occurring only at concentrations of AFP equal to or greater than 300 μg/ml. In the absence of stimulants, ^{3}H-TdR incorporation of cell cultures containing AFP did not differ significantly from the medium controls. This would appear to indicate a lack of non-specific cytotoxicity or mitogenicity by AFP on the cultured lymphocytes.

The effect of the mitogen dose on the inhibition of lymphocyte stimulation by AFP

Next, experiments were performed to determine whether increasing concentrations of PHA would overcome the inhibitory effect of AFP observed in Table 1. Three different AFP preparations at a concentration of 200 μg/ml were tested as to their effect on the mitogenic response to doses of PHA ranging from 0·03 to 30 μg/ml. The results in Fig. 1 demonstrate that increasing concentrations of PHA do not reverse the suppressive effect of AFP on PHA-induced lymphocyte responses. On the contrary, AFP is actually more suppressive on PHA-stimulated DNA synthesis at supra-optimal concentrations of of the mitogen. It should be noted that the peak level of mitogenic response to PHA elicited by 1 μg/ml of PHA did not alter in the presence of albumin or inhibitory concentrations of AFP.

Lack of interaction between AFP and mitogen and thymidine

To exclude physical interaction between AFP and purified leucoagglutinin (LA) as a likely cause of the observed suppression, possible binding of ^{125}I-labelled AFP to LA, coupled to Sepharose 4B, was analysed. As shown in Table 2 there was no detectable retention of AFP on the LA-Sepharose column

TABLE 1. Dose-response effects of AFP on T and B lymphocyte proliferation *in vitro**

AFP concentration (μg/ml)	Staphylococci (bacteria/ml)†		PHA (μg/ml)†		MLC‡	
	5×10^7	10^8	0·1	1·0	Responder cell alone	Allogeneic mixture
	^{3}H-TdR incorporation (mean ± s.e.)					
—	10,826±645	9402±372	16,958±2828	55,250±3912	2688±258	13,313±336
600	n.d.	n.d.	n.d.	n.d.	3621±378	2978±418
300	12,871±960	13,064±498	7839±169	32,279±446	6159±1013	6598±264
150	20,478±1235	15,016±1204	8057±679	34,633±1376	4052±718	10,312±1187
75	18,764±1472	14,555±687	8634±368	38,180±427	3151±420	11,247±81
36	16,238±512	12,145±836	10,411±956	41,731±1960	3225±676	16,408±1395
18	10,331±1186	12,427±792	11,866±495	46,644±1113	2851±667	15,264±1322

* All lymphocyte cultures for this experiment refer to cells from a single donor.

† 72-hr serum-free lymphocyte cultures stimulated with formalin-killed *Staphylococcus aureus* strain Cowan I (with a high content of protein A), or with purified PHA in the presence of 200 μg/ml foetal-derived AFP, or in the absence of added protein (medium control). See the Materials and Methods section for details of the culture conditions.

‡ 96-hr one-way mixed lymphocyte cultures in serum-free medium. ^{3}H-TdR incorporation of irradiated (200R) stimulator cells alone was 1053±102 cpm.

n.d. = Not done.

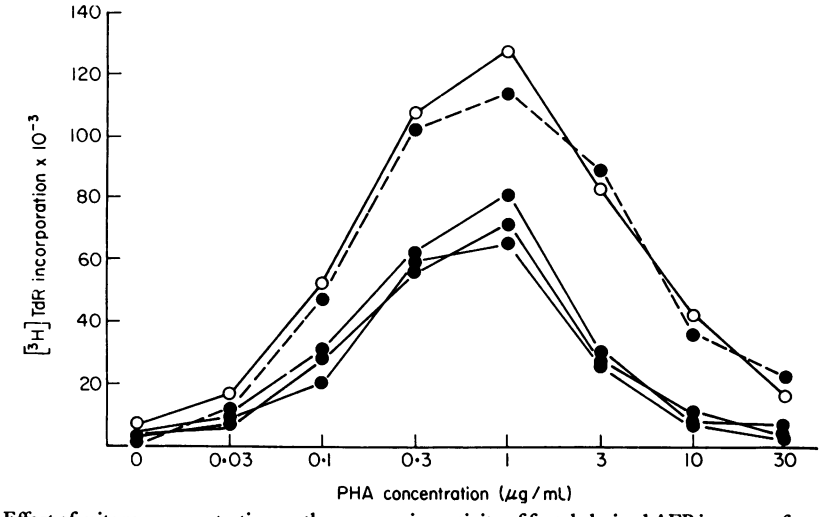

FIG. 1. Effect of mitogen concentration on the suppressive activity of foetal-derived AFP in serum-free medium. Human peripheral blood lymphocytes were stimulated with 1 μg/ml PHA in the presence of 200 μg/ml of 3 different foetal-derived AFPs (●———●), 200 μg/ml of foetal-derived albumin (○———○), or just medium alone (●- - -●). Each point represents the arithmetic mean of triplicate 48-hr cultures. The mean standard error of the group was 5·2%. Similar degrees of AFP-induced suppression were observed in 72-hr mitogen stimulated cultures.

thus indicating that the reaction between human AFP and the phytomitogen to be either very weak or lacking. Purified carcinoembryonic antigen (CEA), known to bind to LA (Hammarström *et al.*, 1975), was included here as a positive control demonstrating the actual presence of LA on the Sepharose beads.

We also tested for the possible binding of AFP to thymidine by incubating 50,000 cpm of tritiated thymidine with 200 μg of AFP, 200 μg of human serum albumin or no protein in 1 ml of PBS. After incubation for 2 hr or overnight the protein was precipitated by 67% saturation with ammonium sulfate, and the radioactivity in the supernatant and precipitate was counted. 6·0 and 1·3% of the total radioactivity was found in the AFP precipitates in two separate experiments. Similar values were obtained in blank tubes or when albumin was used as a control, instead of AFP, thereby excluding the possibility that the inhibition of PHA responses could be due to trivial competitive binding of the added tritiated thymidine by AFP.

Variations in the suppressive activity of individual AFP preparations

Several individually isolated AFP preparations at a concentration of 200 μg/ml were compared for their suppressive activity on PHA-induced lymphocyte transformation. The results are summarized in Table 3. The degree of suppression can be seen to vary considerably with individual preparations. One AFP preparations (AFP-10) isolated from a foetal extract entirely lacked suppressive activity at 200 μg/ml. AFP-10 remained non-suppressive when the concentration in the cultures was increased to 400 μg/ml (data not shown), or if pre-cultured with responding lymphocytes for 16 hr prior to the addition of mitogen, or even after extensive dialysis in human pregnant serum or cord serum (see Table 4).

Influences of culture conditions on the suppressive activity of AFP

An analysis of the conditions of testing *in vitro* revealed that human AFP was more suppressive in serum-free culture conditions. The data in Fig. 2 show that the same AFP preparation which was highly suppressive at 200 and 100 μg/ml in serum-free culture, was considerably less inhibitory in cultures containing 10% pooled AB positive human serum, and was not suppressive in 5% foetal calf serum supplemented medium. Equivalent concentrations of albumin produced a dose-related enhancement of

TABLE 2. Lack of interaction between human AFP and leucoagglutinin (LA)

	Radioactivity bound to particles (%)	
	LA-Sepharose	Sepharose
AFP	1·6±0·2*	2·0±0·1
CEA†	91·0±1·3	1·3±0·1

* Mean and standard deviation of 3 determinations.
† Carcinoembryonic antigen.

TABLE 3. Summary of the suppressive effects of individual human AFP preparations on PHA-induced lymphocyte transformation*

AFP source	^{3}H-TdR incorporation†			
	AFP (200 μg/ml)‡	No AFP	No PHA	Suppression %
	mean cpm±s.e.			
AFP-1 hepatoma-derived	13,702±993	23,033±628	395±49	38
AFP-2 Foetal-derived	11,253±3787	22,755±1213	1187±290	51
AFP-3 Foetal-derived	1667±236	33,314±899	1023±69	95
AFP-4 Foetal-derived	192±109	13,192±32	577±143	99
AFP-5 Foetal-derived	22,212±942	32,176±614	388±7	31
AFP-6 Foetal-derived	84,011±2096	114,291±10,116	4466±599	27
AFP-7 Foetal-derived	70,791±4012	114 291±10,116	4111±266	38
AFP-8 Foetal-derived	66,119±972	114,291±10,116	4792±1052	43
AFP-9 Foetal-derived	42,645±982	78,515±2481	670±110	46
AFP-10 Foetal-derived	37,132±463	29,181±136	1233±347	0

* Results shown with AFP preparations 1–5, 9, 10 represent seven individual experiments with different lymphocyte donors. The effects of AFPs 6–8 were tested simultaneously on lymphocytes from a single donor.
† 48-hr lymphocyte cultures stimulated with 1 μg/ml PHA in serum-free medium.
‡ Final concentration of purified AFP in the cultures.

TABLE 4. Demonstration of suppressive (AFP-2) and non-suppressive (AFP-10) foetal-derived human AFP preparations on PHA-induced lymphocyte transformation*

Control (media)	AFP-2 (200 μg/ml)†	AFP-10 (200 μg/ml)†	AFP-10 added to cultures:			
			16 hr before addition of PHA	After dialysis in:		
				NHS	PHS	CS
			^{3}H-TdR incorporation (mean cpm±s.e.)			
24,864±1323	12,305±2090	34,425±2042	47,797±5324	42,149±303	40,672±2508	36,428±5165

* 48 hr lymphocyte cultures stimulated with 1 μg/ml PHA in serum-free medium.
† Final concentration of purified AFP in the cultures.
‡ AFP-10 was added to lymphocyte cultures in a concentration of 200 μg/ml 16 hr prior to the addition of mitogen, or after 48 hr dialysis in normal human serum (NHS), pregnant (mid-term) human serum (PHA), or cord serum (CS).

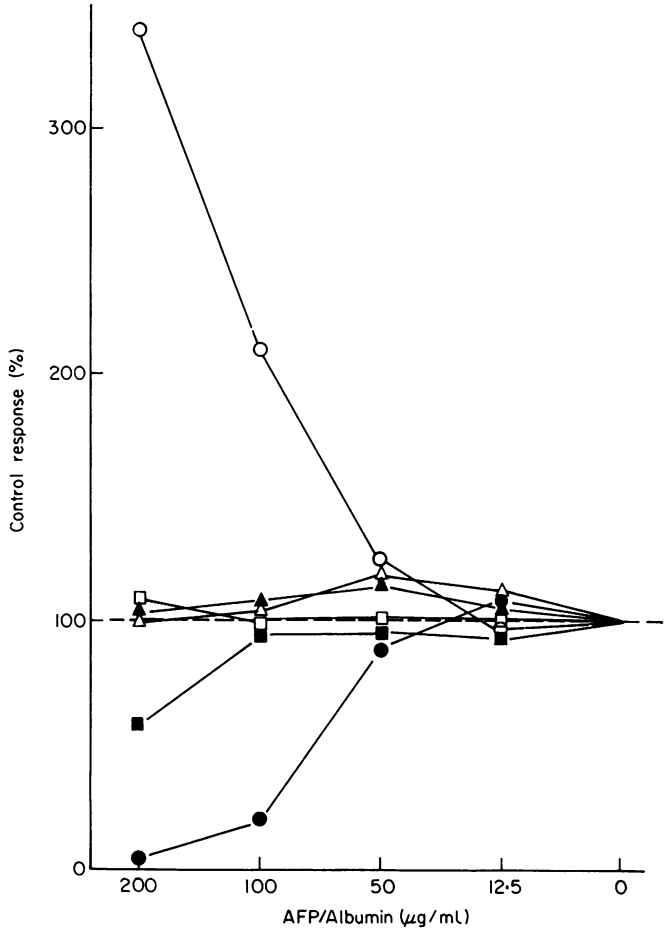

FIG. 2. Influence of culture conditions on AFP-induced suppression of PHA responses. Purified foetal-derived AFP or an equivalent concentration of adult human serum albumin (Alb) was added at the initiation of mitogen (1 μg/ml PHA) stimulated lymphocyte cultures supplemented with 10% pooled AB positive human serum (NHS), 5% foetal calf serum (FCS), or without serum (SF). Each point represents the mean ^{3}H-TdR incorporation of triplicate cultures expressed as a percentage of control cultures (dotted line) in the absence of AFP or albumin. AFP: (●) SF; (■) NHS and (▲) FCS. Albumin: (○) SF; (□) NHS and (△) FCS.

PHA responses in serum-free medium, but did not noticeably affect the mitogen-stimulated DNA synthesis in cultures containing isologous or heterologous serum.

DISCUSSION

In the present investigation we have studied the effects of human foetal- and hepatoma-derived AFP on the proliferative capacities of human B and T lymphocytes to mitogens and alloantigens *in vitro*. These studies demonstrate that purified AFP, in concentrations which effectively suppress PHA- and alloantigen-induced T lymphocyte transformation, fails to influence the mitogenic response of human B cells to *Staphylococcus aureus* bacteria. Thus, in parallel with findings reported earlier in the murine AFP system (Murgita & Wigzell, 1976; Peck *et al.*, 1978; Murgita *et al.*, 1977a), human AFP seems capable of exerting selective inhibitory action on certain T cell functions *in vitro* without directly affecting B cell responses.

PHA is a phytomitogen known to bind to a number of serum glycoproteins (Morse, 1968). Thus, the AFP-induced suppression of PHA responses could conceivably be due to a physical interaction between AFP and PHA interfering with the capacity of the mitogen to stimulate lymphocytes. The absence of an alteration in the peak mitogenic response to PHA in the presence of an inhibitory concentration of AFP (Fig. 1), and the demonstrated lack of binding of radiolabelled AFP to leucoagglutinin (Table 2) would appear to suggest, however, that a direct interference with mitogen is an unlikely explanation for the suppression by AFP. Another possible mechanism through which AFP could prevent PHA-induced lymphocyte stimulation might involve competition with mitogen for surface receptors on target lymphocytes. In this case the inhibition should at least be partially overcome by increasing the concentration of PHA, yet as has already been noted in Fig. 1 this is not the case. In fact, increasing the dose of PHA not only failed to reverse the inhibition, but three different foetal-derived AFP preparations were shown to be more suppressive at supra-optimal concentrations of the mitogen. These findings strongly suggest that human AFP inhibits PHA-reactive T cells by a mechanism(s) other than binding interference or competition with mitogen for cell surface receptors.

An unexpected finding was that heterologous, and to a lesser extent isologous, sera used to supplement the culture medium interfered with the suppressive action of human AFP on PHA responses. Thus, the strong inhibitory effect by AFP observed in mitogen-stimulated serum-free lymphocyte cultures was significantly weaker in parallel cultures containing 10% pooled AB positive human serum, and was usually undetectable in the presence of 5% foetal calf serum. This effect was not detectable in other AFP systems, however, and mouse AFP, for instance, is highly suppressive *in vitro* in the presence of foetal calf serum (Murgita & Tomasi, 1975a,b; Murgita & Wigzell, 1976). The actual reason(s) for the serum effect is therefore obscure. However, it is known that isologous, and particularly heterologous, sera commonly used as supplements to promote growth in tissue culture mediums exert complex stimulatory or inhibitory effects on lymphocyte transformation in *vitro*. Furthermore, the results in Fig. 2 show that the effects the human serum can be overcome by increasing the concentration of AFP. Therefore, in spite of a possible competitive interference by serum factors, AFP-induced immunosuppression may predominate in the human foetus and newborn environment, as well as within the microenvironment of disease processes associated with AFP re-synthesis (Abelev, 1971; Adinolfi *et al.*, 1975), due to effective local concentrations of AFP.

The apparent dissociation between the effects of AFP on PHA- and MLR-reactive T cells noted in this study (Table 1) may reflect a relative difference in sensitivity between these two types of T cell-mediated reactions to AFP-induced suppression. Dose-response experiments showed that the amounts of AFP required to suppress PHA responses were lower than those necessary to inhibit human MLR. Comparisons of the effects of AFP over a wide range of concentrations on other T cell-mediated reactions may reveal additional dichotomies in the *in vitro* immunoregulatory properties of AFP. Quantitative studies in the murine system have already demonstrated that helper T cells for antibody responses to T cell-dependent antigens *in vitro* are more sensitive to suppression by AFP than are the proliferating T cells in MLR involving entire major histocompatibility complex (MHC) differences (Murgita & Tomasi, 1975a & b; Murgita, 1976). However, T cells reacting selectively in primary and secondary MLR towards defined MHC I region differences are highly sensitive to AFP-induced suppression (Peck *et al.*, 1978). The relative insensitivity of human MLR to AFP-induced suppression, in comparison to PHA responses, might indicate that human AFP is capable of exerting selective inhibitory effects only on some sub-classes of T lymphocytes. Evidence does exist for functionally distinct subpopulations of human T cells (Sasaki *et al.*, 1975), and several reports suggest that mitogens and alloantigens may activate different T cell subpopulations in many species (Andersson & Häyry, 1975; Stites, Carr & Fudenberg, 1974; Colley, Shih Wu & Waksman, 1970). Moreover, there is some evidence to suggest that AFP can indeed exert differential effects on various proliferative T cell functions. For example, purified murine AFP effectively inhibits the response of T lymphocytes to PHA, but less to Con A (Murgita & Wigzell, 1976), two T cell mitogens thought to stimulate, in part, different subclasses of thymus-derived lymphocytes (Stobo & Paul, 1973). In the human system Gupta & Good (1977) have shown that hepatoma-derived AFP can cause a dose-related increase in 'active' T cell rosettes, but at the same time markedly

depress PHA responses. These findings are consistent with our suggestion (Peck *et al.*, 1978) that AFP possesses both lymphocyte-suppressive and lymphocyte-activating properties which are directed towards T lymphocyte targets, which are functionally distinct.

It has been shown by Lester, Miller & Yachnin (1976), as well as in the present study, that individual human AFP preparations, isolated by the same procedure and tested *in vitro* at equivalent protein concentrations, elicit widely different degrees of suppression. The exact reason(s) for this variation is not clear. One possibility supported by the recent findings of Lester *et al.* (1976) is that molecular species of AFP which differ in their chemical properties (Gustine & Zimmerman, 1973) may also differ in their functional, i.e. immunoregulatory, properties. The net suppressive activity of a given AFP preparation could therefore reflect the relative proportions of suppressive versus inactive molecular variants within the preparation. Also, variable degrees of molecular degradation may occur during purification which may adversely affect the protein's functional ability. For example, purification procedures commonly used to isolate HCG (Muchmore & Blaese, 1977), and CFA (Fuks *et al.*, 1974) can lead to the alteration of attached carbohydrate moieties. It has been suggested that the potentially vulnerable sialic acid residues in the AFP molecule are essential for AFP-induced immunosuppressive activity in the murine system (Zimmerman, Voorting-Hawking & Michael, 1977). Therefore, varying degrees of chemical alteration in the AFP molecule itself, incurred during its purification, could conceivably be the cause, at least in part, of the differences in suppressive activity. The alternative possibility that chemical degradation may somehow induce immunosuppressive properties in an otherwise inert AFP molecule is untenable. Thus, the selective removal of AFP from mouse amniotic fluid (Murgita & Tomasi, 1975b) or pregnant mouse serum (Murgita, 1976) will abrogate their immunosuppressive activities. AFP molecules with intrinsic suppressive properties may, however, act in conjunction with other molecules in a complicated manner. In line with this are the findings that AFP may complex with a number of low molecular weight substances (Nunez *et al.*, 1974), some of which have been suggested as immunosuppressive agents (Adcock *et al.*, 1973) although with notable reservations (Schiff, Mercier & Buckley, 1975; Morse *et al.*, 1977). Such hypothetical suppressive AFP-peptide complexes could play an important immunoregulatory role in the maternal-foetal environment where low molecular weight inhibitory factors have been shown to exist (Wolf, Lomnitzer & Rabson, 1977; Olding, Murgita & Wigzell, 1977). We failed in the present study however to re-associate such a putative low molecular weight suppressive moiety with a non-inhibitory AFP preparation by extensive dialysis in human pregnant serum or cord serum. Moreover, the suppressive activity of murine AFP is retained on exposure to low pH and high salt, followed by exhaustive dialysis (Murgita & Tomasi, 1975b), conditions known to favour the removal of the suppressive peptide from its carrier alpha-globulin (Occhino *et al.*, 1973). It therefore appears unlikely that the suppressive effect of AFP *per se* is merely caused by its carrying a hypothetical immunosuppressive small molecule which may be lost during the purification of AFP.

In conclusion, certain functions of human T lymphocytes, in contrast to B cells, are susceptible to inhibition by homologous AFP *in vitro*. Differences in sensitivity to inhibition suggested that human T cells like murine T cells vary according to sub-groups as to sensitivity to AFP-induced suppression. The relationship between the present findings, the ability of murine AFP to induce suppressor T cells *in vitro* (Murgita *et al.*, 1977b) and the presence of suppressor T cell in newborn mice (Mosier & Johnson, 1975) or children (Olding & Oldstone, 1976; Olding, Murgita & Wigzell 1977) is now an obvious target for further analysis.

This work was supported by a Swedish MRC fellowship and a Quebec MRC grant to Dr R. A. Murgita, an EMBO fellowship to Dr L. C. Andersson, by grants from the Swedish Cancer Society to Dr H. Wigzell, and Swedish MRC grants nos. 13X-03556, 13P-05037 to Dr H. Bennich.

We thank Dr E. Ruoslahti for supplying us with samples of purified human AFP, Dr B. Kjessler at the Akademiska Sjukhuset in Uppsala for helping us obtain cord serum, and Dr Eva Engvall for performing the studies on the interaction between AFP and leucogglutinin. We also especially wish to thank Angela C. Murgita for her fine technical assistance and help in the preparation of this manuscript.

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