CELLULAR AND GENETIC RESTRICTIONS IN THE IMMUNOREGULATORY ACTIVITY OF ALPHA-FETOPROTEIN

II. Alpha-Fetoprotein-Induced Suppression of Cytotoxic T Lymphocyte Development*

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Alpha-fetoprotein (AFP),[1] a normal component of fetal and newborn sera, has been shown in both the human and murine systems to exert selective suppressive effects on various functions of thymus-derived (T) lymphocytes, including T-cell-dependent antibody synthesis, T-cell mitogenic responsiveness, and T-cell-mediated allogeneic reactivity (1–9).[2] In addition, recent reports have also revealed that under special circumstances AFP may exert a supportive influence on in vitro cell growth (9–11) with one important manifestation of this activating property being the in vitro induction of suppressor T cells (12).

We have undertaken a study into the possible regulatory influence of AFP on the recognitive and effector phases of T-cell-mediated allogeneic immune reactions utilizing the in vitro mixed leukocyte culture (MLC) and cell-mediated lympholysis (CML) assays. In a preceding paper (9), we presented evidence that fetal-derived AFP can exert major differential effects on both the primary and secondary MLC responses of purified T lymphocyte subpopulations ranging from strong suppression to occasional enhancement. Genetic analysis revealed that T-cell proliferation dependent on the recognition of major histocompatibility complex (MHC) I region gene determinants was actively suppressed, whereas proliferative responses induced by MHC serologically-defined (SD) region histoincompatibilities and I region-independent non-MHC differences were generally unaffected.

In view of these findings, one might predict that AFP would fail to suppress the development of cytotoxic T lymphocytes (CTLs) in allogeneic reactions, since the generation of effector T cells appears for the most part to be genetically

* Supported in part by the Swedish Cancer Society and National Institutes of Health grant AI 13485-02.

‡ Recipient of a Swedish Cancer Society Fellowship.

[1] *Abbreviations used in this paper*: AFP, alpha-fetoprotein; CML, cell-mediated lympholysis; CTLs, cytotoxic T lymphocytes; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MLC, mixed leukocyte culture; NMS, normal mouse serum; SD, serologically-defined (as the K and D regions of the mouse MHC); [^{3}H]TdR, tritiated thymidine.

[2] R. A. Murgita, E. A. Goidl, S. Kontiainen, R. C. L. Beverely, and H. Wigzell. 1978. Adult murine T cells activated in vitro by alpha-fetoprotein and naturally occurring T cells in newborn mice: identity in function and cell-surface differentiation antigens. *Proc. Natl. Acad. Sci. (U.S.A.)*. In press.

controlled by determinants encoded for by genes of the MHC SD regions. However, we present in this report results indicating that AFP can actively inhibit the in vitro generation of CTLs even in the very same strain combinations in which AFP has little or no suppressive effect on the proliferative phase. Strain combinations were also found in which the CTL development proved refractive to the suppressive activity of AFP; however, such reactions always contained, in addition to the MHC difference(s), certain undefined lymphocyte-activating non-MHC loci differences. Thus, the genetic relationship between responding and stimulating cells largely predetermines whether or not AFP will inhibit the generation of CTLs. These data, together with those of the previous paper (9), allow us to speculate about possible mechanisms for AFP-induced suppression of T-cell-mediated reactions.

Materials and Methods

Mice. Inbred strains of mice used in this study and maintained in this laboratory are AQR, B10.A, B10.BR/cd, B10.D2/n, B10.G, B10.S, B10.S(7R), B10.T(6R), CBA/H, CBA-H-2^{ka}, C57BL/6Bom, and DBA/2. Breeding pairs of AQR were kindly provided by Dr. E. Simpson (Transplantation Biology, Clinical Research Center, Harrow, Middlesex, England). The mutant strain CBA-H-2^{ka} was obtained from Dr. L. C. Andersson (Transplantation Laboratory, University of Helsinki, Helsinki, Finland). Both male and female mice ranging in age from 4 to 24 wk were utilized.

AFP. AFP was isolated and purified as described elsewhere (2, 9).

MLC. The complete protocol for the MLC reactions, including the description of culture medium and preparation of splenic T lymphocytes, has been detailed previously (9). Primary MLC were supplemented with 150 μg/ml AFP or an equivalent amount of normal mouse serum (NMS).

CML Assay. CML were performed according to procedures detailed elsewhere (13) and are basically a modification of the techniques described by Alter et al. (14). The effector cells were generated in primary MLC. The primed lymphocytes were collected from the mixed cultures, pooled, centrifuged, and washed twice in medium supplemented with 2.5% fetal bovine serum (Microbiological Associates, Walkersville, Md).

Lipopolysaccharide (LPS)-stimulated spleen cells or the in vitro grown P815 tumor were used as the target cells. Approximately 36 h before the CML assay, appropriate target cell cultures containing 12.0×10^6 spleen cells in 5.0 ml serum-free medium were established in culture dishes (3002, BioQuest, BBL & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.). Target cells were incubated 2 h then stimulated with 25–50 μg/ml LPS prepared by Dr. J. Andersson (Department of Immunology, Uppsala University, Uppsala, Sweden). At time of assay the target cells were collected, centrifuged, resuspended in 0.4 ml of supernate removed from the centrifuged cells, and labeled 1 h with 400–500 μCi $Na_2{}^{51}CrO_4$. The labeled cells were washed three times in medium.

Cell destruction was performed in round-bottomed Sterilin microtiter plates (Sterilin Ltd., Teddington, Middlesex, England) using various effector cell numbers plus 1.0×10^4 labeled target cells. Cell destruction proceeded 4 h at 37°C, after which time the plates were centrifuged, the supernate collected, and the quantity of released ^{51}Cr determined. Percent cytotoxicity is expressed as

$$\frac{{}^{51}\text{Cr-released}_{(\text{exp. 1})} - {}^{51}\text{Cr-released}_{(\text{spontaneous})}}{{}^{51}\text{Cr-released}_{(\text{maximum})} - {}^{51}\text{Cr-released}_{(\text{spontaneous})}} \times 100\%.$$

Results

The T-cell-mediated cytotoxic reaction is generally dissociated into a recognitive phase, in which the recognition of alloantigens results in the clonal proliferation of specific antigen-activated cells, and a destructive phase, in which activated cells within the responding population demonstrate cytotoxic

activity against cells bearing the sensitizing alloantigens. Genetic studies (15, 16) have revealed that the in vitro development of CTLs apparently requires: (*a*) activation of a hypothesized proliferating helper cell population by alloantigens of the MHC, and (*b*) sensitization of the CTL precursor population by serologically-defined MHC alloantigens, or antigens of loci closely linked to genes encoding for the SD determinants, concurrently present during the activation phase. In the mouse, lymphocyte activation as measured by MLC can be induced by genetic differences associated with the MHC (17, 18) or with loci segregating independently of the MHC (19–22) (referred to as non-MHC loci in the present report). The various regions and subregions comprising the MHC contain genes which code for at least two distinct classes of membrane-bound MLC-activating products. The K and D regions encode for the classical SD determinants, whereas the I region genes encode for the Ia antigens. Both the SD and Ia molecules, besides initiating lymphocyte proliferation, appear to function as target antigens for cytotoxic T lymphocytes in CML (14, 23). Two MLC-stimulating non-MHC antigenic systems of interest for the present investigation are the Mls locus (19, 20) and an as yet undefined system (22). These two non-MHC systems are readily distinguishable from one another through lymphocyte-typing analysis, i.e., Mls locus products induce lymphocyte proliferation only if there is concommitant recognition of MHC I region gene products, whereas the undefined non-MHC system stimulates T lymphocytes directly (22). Neither of the non-MHC alloantigens apparently function as target antigens for CTLs. However, studies by Schendel and Bach (24) and Wagner et al. (25) have indicated that the presence of non-MHC lymphocyte-activating alloantigens during the proliferative phase can substitute for the MHC differences which normally induce the MLC necessary for the generation of CTLs. These four classes of determinants, together with their presently known functions, are listed in Table I. In addition, Table I indicates how these four systems are further delineated by the differential activity AFP exerts on T-cell proliferation induced by each system (9). Note that while AFP generally suppresses proliferative reactions involving I region recognition (including anti-Mls locus responses), it has little or no effect on reactions against SD region determinants and actually has the potential to enhance reactions involving the undefined non-MHC system.

AFP-Induced Suppression of the In Vitro Development of CTLs. Generation of mature CTLs in the absence or presence of AFP has been investigated using congenic resistant partner strains possessing a variety of MHC-associated genetic differences. One representative reaction involving a whole MHC difference, the response of purified B10.BR splenic T lymphocytes against X-irradiated B10.D2/n spleen cells, is depicted in Fig. 1. The kinetics of primary MLC activation revealed the expected suppression of the proliferative responses in cultures containing AFP (Fig. 1 A). After peak tritiated thymidine ([^{3}H]TdR) incorporation, the NMS-generated and the AFP-generated cells were examined in CML for their capacity to elicit cytolysis. Whether these two populations of effector cells are compared on a culture to culture basis (Fig. 1 B) or on a cell to cell basis (Fig. 1 C), similar results were obtained: NMS-generated blasts specifically lysed the LPS-stimulated B10.D2/n target cells, whereas the AFP-generated cells exhibited no (or only marginal) cytolytic activity.

TABLE I

List of Important Histocompatibility Antigenic Systems Influencing the Control of the T-Cell-Mediated Cytotoxic Reaction and the Effects of AFP on the Proliferative Phase

Antigenic system*	Induction of MLC activation*	Effects of AFP on MLC‡	Target antigen in CML§
Ia	+++	Suppression	+/−
SD	++	No effect or slight suppression	+++
Mls-Locus	+	Suppression	−
Non-MHC	++	No effect or enhancement	−

* Klein (17), Shreffler and David (18), Festenstein et al. (19, 20), and Peck et al. (22).
‡ Peck et al. (9).
§ Nabholz et al. (23), Alter et al. (14), and Peck et al. (13).

Data presented in Fig. 1 C also indicate that the addition of AFP to the CML assay failed to alter the cytotoxic response of the CTLs. No significant differences in the percent ^{51}Cr-release values over the ratios of effector to target cells tested was observed between the CML assays containing AFP and those not containing AFP. This would indicate that the killing mechanism per se of mature CTLs is not altered significantly by AFP, and, at the same time, AFP does not block or interfere with recognition of the target cell antigens.

MHC recombinant strains possessing limited genetic differences permit the study of the effects of AFP on the generation of CTLs in allogeneic reactions induced by individual SD regions of the MHC. Data presented in the previous report (9) suggested that AFP acts primarily on a subset of T lymphocytes which demonstrate strong proliferative reactivity against products of the MHC I region genes, while failing to inhibit proliferative responses against isolated SD region differences. It might be predicted, therefore, that AFP should have little or no effect on the generation of CTLs in such genetic situations. To test this possiblity, a number of strain combinations possessing isolated MHC SD region incompatibilities were examined to determine if AFP could inhibit CTL development. Results presented in Table II show the effects of AFP in the generation of CTLs in three selected reactions directed specifically against known SD region products. In each combination, AFP clearly inhibited the development of killer T cells, as measured in CML, despite the fact that cell proliferation, as measured by [^{3}H]TdR incorporation, proved similar in cultures containing AFP and in the control cultures. These three strain combinations represent three distinct genetic situations. The first combination, AQR anti-B10.A, is an anti-K region reaction. Primed lymphocyte-typing analysis suggests that the stimulating alloantigen recognized by the responding cells is associated with the SD specificity H-2.23. Likewise, the reaction of B10.S towards B10.S(7R) appears to be induced by the MHC D region-associated alloantigen defined by the two specificities H-2.4 plus H-2.40 present on the SD molecule (A. B. Peck, unpublished data). Reactivity between strains CBA-H-2^{ka} and CBA/H is believed to result from a gain-loss mutation associated with the MHC K region. Thus, lymphocyte proliferation and cytotoxicity are most

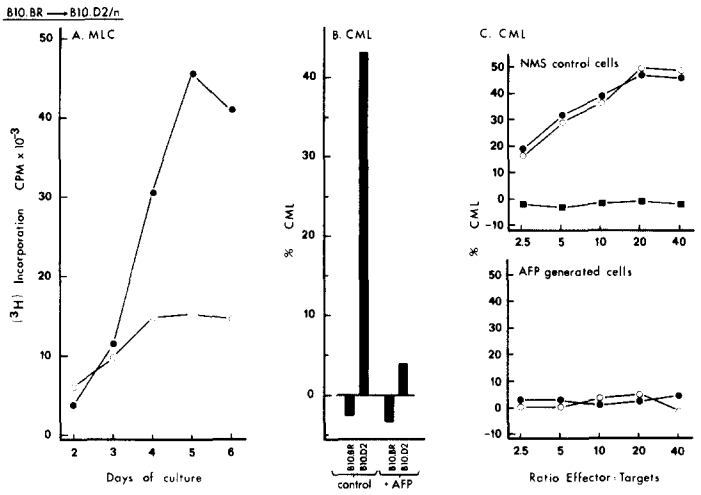

FIG. 1. Suppression of the in vitro generation of CTLs by AFP in a reaction involving a whole MHC haplotype difference. A. Kinetics of MLC activation of purified B10.BR splenic T lymphocytes stimulated with X-irradiated B10.D2/n spleen cells in the absence (●——●) or presence (○——○) of 150 μg/ml AFP. B. Comparison on a culture to culture basis of the cytotoxic reactivity for B10.BR anti-B10.D2/n cells generated in the presence or absence of AFP. After peak [^{3}H]TdR incorporation, equal portions of the NMS-generated and AFP-generated cell cultures were tested in CML on 1.0 × 10^4 ^{51}Cr-labeled, LPS-stimulated B10.BR or B10.D2/n blast cells. Control reactions contained 0.37 × 10^6 blasts and AFP-generated reactions contained 0.24 × 10^6 blasts. C. Comparison on a cell to cell basis of the cytotoxic reactivity for B10.BR anti-B10.D2/n cells generated in the absence or presence of AFP. The activated cells generated in NMS supplemented control cultures and in the AFP-containing cultures were collected and standardized to equal numbers of blast cells, then tested for cytotoxic activity at various numbers, ranging from 0.025 × 10^6 to 0.4 × 10^6 blasts, on 1.0 × 10^4 ^{51}Cr-labeled, LPS-stimulated B10.D2/n blasts in the absence (●——●) and presence (○——○) of 150 μg/ml AFP. No cytolytic activity occurred on B10.BR target cells (■).

probably induced by the parent-mutation strain-defined SD specificity H-2.60 (26). AFP, therefore, clearly possesses the capacity to inhibit CML generated specifically against defined SD alloantigens.

Lack of Suppression by AFP in the Generation of CTLs in Reactions against Strains DBA/2 and B10.G. One extensively used system in the study of T-cell-mediated cytotoxicity is the activation of lymphocytes with cells carrying the MHC *d* haplotype, particularly from DBA/2, followed by testing for cytolysis on the in vitro maintained P815 tumor line. The recent studies of Sheppard et al. (10) have indicated that AFP does not inhibit the generation of CTLs in such a reaction. We have also noted similar results using either C57BL/6 or CBA/H as the responding strain against DBA/2, as shown in Table III. Of major interest here is the fact that DBA/2 cells have been shown previously (9) to carry non-MHC alloantigen systems to which the resulting lymphocyte activation proved refractive to the inhibitory activity of AFP. It is not surprising, therefore, that both the NMS-generated cells as well as the AFP-generated cells elicited strong CML on P815 tumor targets or LPS-stimulated DBA/2 target blasts.

In a similar manner, reactions against strain B10.G have also been found to

TABLE II

AFP-Induced Suppression of CTL Generation in Strain Combinations Possessing Isolated MHC SD Region Differences

Responding strain	Stimulating strain	Genetic differences	Culture	[^{3}H]TdR incorporation* Mean cpm ± SD	Target cells‡	CML§
						%
AQR	B10.A	K Region	NMS	18,650 ± 487	AQR	−5.4
					B10.A	28.4
			+ AFP	19,035 ± 777	AQR	−5.2
					B10.A	−1.8
B10.S	B10.S(7R)	D Region	NMS	27,902 ± 2,406	B10.S	−3.6
					B10.S(7R)	26.4
			+ AFP	25,328 ± 422	B10.S	−10.8
					B10.S(7R)	−5.3
CBA-H-2^{ka}	CBA/H	SD Mutation	NMS	41,440 ± 546	CBA-H-2^{ka}	−2.8
					CBA/H	24.5
			+ AFP	37,156 ± 213	CBA-H-2^{ka}	−3.7
					CBA/H	6.5

* [^{3}H]TdR Incorporation measured at time of CML assay after a 4-h pulse time.

‡ Target cells = LPS-stimulated spleen cells. Spontaneous release values ranged from 8 to 32% of the maximum release.

§ Ratio of effector to target cells = 30:1.

be refractive to AFP-induced suppression of CTL development. This is seen whether in strain combinations possessing SD + I region incompatibility, as shown in Table III for the reaction AQR anti-B10.G, or in strain combinations possessing isolated SD region differences, as shown in Fig. 2 for the reaction B10.T(6R) anti-B10.G. Although B10.G is generally considered an MHC congenic resistant partner strain of C57BL/10, its phenotype clearly shows the presence of the homozygous *tf* gene which C57BL/10 does not (17). B10.G, therefore, must carry a large chromosomal segment outside of MHC which was also derived from the MHC donor strain. The fact that AFP failed to suppress the in vitro generation of CTLs suggests that, similar to DBA/2, B10.G possesses a lymphocyte-stimulating non-MHC alloatigenic system. This concept is now supported further by both primed-lymphocyte typing and newborn-thymus reactions (unpublished data).

A few interesting points concerning the differential effects of AFP in CML reactions of AQR anti-B10.A (Table II), AQR anti-B10.G (Table III), and B10.T(6R) anti-B10.G (Fig. 2) can be drawn from a genetic analysis of the strains involved. Strain AQR utilized in the present study was originally derived from a cross between (T138 × B10.A)F_1 and C57BL/10. The recombinant strain was subsequently backcrossed four times to C57BL/10 before intercrossing. AQR, therefore, carries a considerable portion of the C57BL/10 genome even though it is not a full congenic resistant partner strain. Similarly, strain B10.T(6R) was derived as an MHC recombinant between B10.A and a *tf-T* (tufted-Brachyury) stock, but still carries both markers outside of the MHC. In contrast, B10.G was derived from a noninbred stock which also carried the two

TABLE III

Failure of AFP to Suppress the in Vitro Generation of CTLs in Reactions against Strains DBA/2 or B10.G

Responding strain	Stimulating strain	Genetic differences	Culture	Target cells*	% CML‡
C57BL/6	DBA/2	MHC, Mls, Non-MHC	NMS	C57BL/6	−4.6
				DBA/2	62.7
			+ AFP	C57BL/6	1.9
				DBA/2	71.8
CBA/H	DBA/2	MHC, Mls, Non-MHC	NMS	P815	82.4
			+ AFP	P815	88.2
AQR	B10.G	MHC I,S,G,D Regions	NMS	AQR	−9.8
				B10.G	22.8
			+ AFP	AQR	−13.4
				B10.G	24.3

* Target cells = LPS-stimulated spleen cells or the in vitro maintained P815 tumor line.

‡ Ratio of effector to target cells = 12.5:1 for the reactions C57BL/6 anti-DBA/2 and CBA/H anti-DBA/2, or 30:1 for the reaction AQR anti-B10.G.

markers, *tf* and *T*, and, as stated above, still carries the *tf* marker genes. While the C57BL/10 background is normally a silent background in in vitro lymphocyte activation, this genetic information indicates that these three strains most likely possess genetic material of non-C57BL/10 origin outside of, but linked to, the MHC. It is not surprising, therefore, that unexpected lymphocyte activation and activity occurred between these presumed congenic resistant partner strains. Although one might expect that AQR would recognize non-MHC components on both B10.G and B10.A cells, only the reaction against B10.G proved refractive to AFP-induced suppression of CML. Similarly, since both B10.T(6R) and B10.G were derived from strains carrying *tf* and *T* markers, one might expect these two strains to be quite similar throughout chromosome 17 and thereby see the CML reaction of B10.T(6R) anti-B10.G suppressed by AFP in a normal manner. These deviations from the expected probably stem from the fact that AQR is derived in part from B10.A and is nearly congenic with C57BL/10 in the non-MHC background, while the combinations AQR-B10.G and B10.T(6R)-B10.G have major non-MHC genetic differences arising from their independent derivations. Linkage studies are now being conducted to determine if the non-MHC lymphocyte stimulating alloantigenic system associated with B10.G is identical to that of the DBA/2 strain and whether it maps to chromosome 17 (linkage group IX). Note, also, that reactions of AQR cells can be both suppressed by AFP and refractive to the suppressive activity of AFP. This indicates that the effects of AFP are not inherent in the genetics of the responding strain.

The differential effect of AFP on CML, shown in Tables II and III, is a highly reproducible phenomenon and there is no indication that it results from variability in different samples of AFP. Three different preparations of AFP have been utilized in the present study. All experiments except the reactions of CBA-H-2^{ka} anti-CBA/H (Table II) and CBA/H anti-DBA/2 (Table III) were performed with one AFP preparation. Thus, we would conclude that the

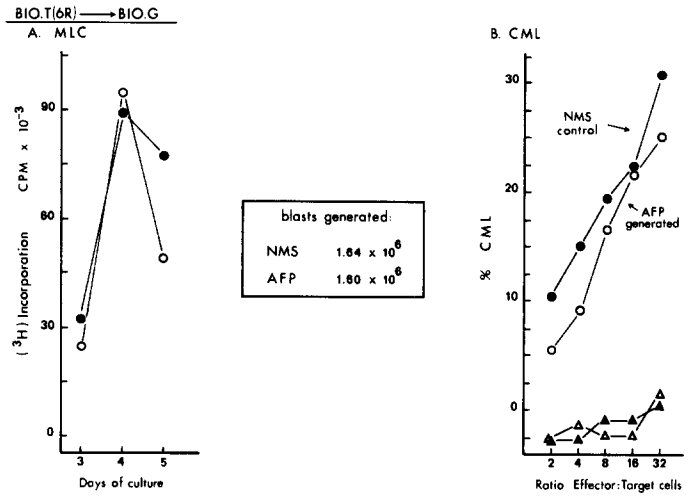

FIG. 2. Lack of an AFP-induced suppression of CTL development in the reaction of B10.T(6R) against B10.G. A. Kinetics of MLC activation of purified B10.T(6R) splenic T lymphocytes responding against B10.G spleen cells in the absence (●——●) and presence (○——○) of 150 μg/ml of AFP. B. CML of NMS-generated (●——●, ▲——▲) and AFP-generated (○——○, △——△) B10.T(6R) anti-B10.G blast cells. After peak [³H]TdR incorporation in MLC, cells from each culture were collected and standardized to equal numbers of blast cells, then tested for cytotoxic activity at various numbers, ranging from 0.02×10^6 to 0.32×10^6 blasts, on 1.0×10^4 ^{51}Cr-labeled B10.G (● or ○) and B10.T(6R) (▲ or △) target cells.

inhibition or lack of inhibition of the in vitro generation of CTLs depends to a large extent on the genetic relationship between responding and stimulating strains.

Inhibition of the AFP-Induced Suppression of CTL Development by the Introduction of Nonsuppressible Anti-Non-MHC Reactions into the Primary MLC. Data obtained thus far indicate that the presence of AFP during the activation phase exerts in general a selective suppressive activity on the subsequent development of CTLs. This inhibition, while independent of the suppressibility of the MLC activation phase (Table II), is dependent on the genetic relationship between the responding and stimulating strains (Table III). For example, in this study we have found that both DBA/2 and B10.G cells, when utilized as the stimulating antigen, are capable of initiating events which circumvent the mechanism(s) of AFP suppression. The possibility that this circumvention results from the presence of non-MHC components is suggested from a comparison of the two reactions B10.BR anti-B10.D2/n (Fig. 1) and CBA/H anti-DBA/2 (Table III). Both reactions are MHC *k* anti-*d* responses; however, CBA/H and DBA/2 differ genetically at MLC-activating non-MHC loci, whereas B10.BR and B10.D2/n are congenic resistant partner strains and considered to be genetically identical at all non-MHC loci.

To test the possibility that the introduction of a nonsuppressible anti-non-MHC reaction present during the priming phase can cause the circumvention of the mechanism of AFP suppression, we have utilized the protocol of a three-cell experiment in which the stimulating antigens are presented to the responding

cells on two different stimulating strains. Purified splenic T lymphocytes from B10.D2/n were stimulated in vitro in the absence and presence of AFP with either X-irradiated B10.BR plus syngeneic B10.D2/n cells or X-irradiated B10.BR plus DBA/2 cells. The response of B10.D2/n against DBA/2, considered a pure anti-non-MHC reaction, is characterized by lymphocyte proliferation but no generation of CTLs (13). Furthermore, the presence of AFP in the B10.D2/n anti-DBA/2 reaction tends to enhance the resulting lymphocyte proliferation (9). On day 6 of primary MLC, the activated cells from each culture were tested in CML for their killing potential. Results, summarized in Table IV, reveal that the addition of DBA/2 cells to the otherwise AFP-suppressible B10.D2/n anti-B10.BR reaction inhibits the suppression and permits the development of CTLs with specificity for the SD determinants of B10.BR.

Discussion

Earlier analysis (9) of the impact exerted by AFP on the recognition and subsequent proliferation of T lymphocytes reacting in MLC against defined histocompatibility alloantigens revealed a highly selective activity in the suppression of lymphocyte responses. In general, AFP inhibited Ly 1^+ T blast cells reacting against I region structures (including reactions against Mls locus products whose recognition apparently requires concommitant recognition of I region gene products), but failed to inhibit Ly 2^+ T cells stimulated by SD alloantigens. Thus, it seemed clear that AFP exerted its suppressive activity in MLC via selective interference with I region triggering systems. However, results of the present study, which has concentrated primarily on the capacity of AFP to suppress the effector phase of the T-cell-mediated cytotoxic reaction, suggests a broader spectrum of regulatory activity. For example, AFP, when present during the primary activation phase of T-cell responses, not only suppressed the subsequent in vitro generation of effective CTLs in strain combinations with I plus SD region differences (Fig. 1), but also in congenic strain combinations possessing only SD region differences where the proliferative phase was unaffected (Table II). If AFP interfered only with I region triggering, then it would have been expected that at least in reactions directed against isolated MHC SD region-associated gene products not only the proliferative but also the cytotoxic phase would have remained refractive to the suppressive activity of AFP.

How AFP suppresses the generation of CTLs remains to be determined, but any discussion concerning a possible mechanism first necessitates a basic understanding of the events which control development of killer T cells in the normal situation. One widely held hypothesis (27) argues that a subpopulation of T cells, the CTL precursor cells, initiates the reaction by first recognizing alloantigens of the MHC SD regions and thereafter differentiating to a poised-CTL receptive to a help-signal. A second subpopulation of T cells, the proliferating helper cell, recognizes and responds against the lymphocyte-activating determinants of the MHC and then, either through cell-cell contact or a soluble factor, collaborates with the poised-CTL pool. It is the T-T cell collaboration which induces the final differentiation of the poised-CTL to an active killer T cell. Such a two-step hypothesis has been further supported by the studies of

TABLE IV

Inhibition of the AFP-Induced Suppression of T-Cell-Mediated Cytotoxicity by Addition of Third Party Stimulating Cells with Non-MHC Genetic Differences

Responding strain	Stimulating strain	Genetic differences	Culture	[^{3}H]TdR Incorporation Mean cpm ± SD	Target cells*	% CML‡
B10.D2/n	B10.BR + B10.D2/n	MHC + None	NMS	10,148 ± 649	B10.D2/n	−6.4
					B10.BR	38.9
			+ AFP	2,491 ± 152	B10.D2/n	−9.2
					B10.BR	−4.1
B10.D2/n	B10.BR + DBA/2	MHC + Non-MHC	NMS	9,878 ± 491	B10.D2/n	−9.6
					B10.BR	42.0
			+ AFP	4,993 ± 335	B10.D2/n	−7.6
					B10.BR	22.9

* Target cells = LPS-stimulated spleen cells. Spontaneous release values are: B10.D2/n 598 ± 22, B10.BR 420 ± 11. Maximum release values are: B10.D2/n 2,689 ± 218, B10.BR 2,159 ± 407.

‡ Ratio of effector to target cells = 40:1.

Plate (28) and Sopori et al. (29) which suggest that the pre-killer T cell must receive two signals, one of which eminates from the helper T cell, before differentiating to a specific effector T lymphocyte.

It is now clear that AFP-induced suppression of CTL development, similar to the AFP-induced suppression of lymphocyte proliferation in MLC (9), is dependent on parameters controlled in part by the genetic relationship between responding and stimulating strains. This is seen in a comparison of the two reactions B10.BR anti-B10.D2/n (Fig. 1) and CBA/H anti-DBA/2 (Table III). Both reactions are MHC *k* anti-*d*, but the latter reaction is also characterized by the presence of numerous MLC-activating non-MHC differences. The fact that inhibition of the generation of CTLs was only effected by AFP in the reaction between the congenic resistant partner strains, B10.BR-B10.D2/n, suggests that the non-MHC component is a responsible factor in circumventing the mechanism of AFP suppression. This concept is strongly supported by the three-cell experiments in which the introduction of a non-MHC lymphocyte-activating difference into the primary MLC of an otherwise AFP-suppressible reaction led to the development of CML activity (Table IV).

In view of the results observed in both the present study and the previous paper (9), three mechanisms of AFP-induced suppression of T-cell-mediated cytotoxicity appear feasible. First, AFP may directly suppress several distinct subpopulations of T lymphocytes, including the Ly 1$^+$ proliferating T-cell responsive against MHC I region-gene products and the CTL precursor cell. Second, AFP may interfere solely with the proliferating helper T-cell response against MHC-associated lymphocyte-activating determinants and in this manner block the T-T cell collaboration necessary for the differentiation of the CTL precursor to a killer cell to occur. Third, AFP may inhibit the production of CTLs by inducing suppressor T cells capable of blocking either directly or indirectly the collaborating T-cell populations. This third possibility receives

indirect support from evidence presented in another test system (12)[2] in which AFP was shown capable of activating Ly 1^+,2 T cells in vitro which efficiently blocked helper T-cell-dependent antibody synthesis.

At present we believe that the key in proposing a mechanism for AFP suppression comes from the observation that the presence of lymphocyte-activating non-MHC determinants can prevent suppression of CTL formation by AFP (Tables III and IV). Studies of Schendel and Bach (24) and Wagner et al. (25) indicate that lymphocyte activation by non-MHC determinants also provides the helper T-cell signal to the poised-CTL or CTL precursor cell. Thus, it would seem most probable that the regulatory activity of AFP, by whatever means the actual suppression is elicited, lies at the level of the helper T-cell signal. This point is presently being investigated.

In conclusion, AFP is endowed with selective ability to abrogate the in vitro generation of CTLs in allogeneic reactions. This selective activity not only indicates a potential in vivo relevance of AFP as an immunoregulatory factor, but also provides us with a new approach in the dissection of the T-cell-mediated cytotoxic reaction. Equally important, however, may be the possible use of AFP as a selective reagent in obtaining specific, alloantigen-reactive T-cell subsets.

Summary

Alpha-fetoprotein (AFP), a major component of fetal and newborn sera, was shown to exert significant immunosuppressive activity on the in vitro generation of cytotoxic T lymphocytes (CTLs). This suppression proved independent of the suppressibility of the mixed leukocyte culture activation phase, since strain combinations whose proliferative responses were refractive to AFP-induced suppression also failed to develop demonstrable CTLs in the presence of AFP. Several strain combinations were also found in which normal generation of CTLs occurred in cultures containing AFP. This refractive nature correlated with the presence of nonsuppressible lymphocyte-stimulating alloantigenic systems on the stimulating cell population. These data provide the basis for proposing several possible mechanisms for AFP-induced suppression of T-cell-mediated cytotoxicity, as well as suggesting that the primary target of this suppression is the proliferating helper T cell precommited to respond towards the major histocompatibility complex-associated lymphocyte-activating determinants.

The authors wish to thank Ms. Gunilla Andersson for her excellent technical help and Ms. Birgitta Ehrsson for her help in the preparation of this manuscript.

Received for publication 13 February 1978.

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