ALPHA-FETOPROTEIN SYNTHESIS BY MURINE LYMPHOID CELLS IN ALLOGENEIC REACTIONS*

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The effects of the graft vs. host disease (GVH)[1] on murine immune functions are currently under intensive study. A recent report (1) suggests that there is a heightened immune responsiveness during the 1st wk of the GVH reaction followed subsequently by immunosuppression. These time differences in immune reactivity may explain, at least in part, earlier contradictory reports on the level of the antibody response (2, 3) and the effects on tumor growth (4–8) of GVH reactions. In addition, the late immunosuppression has been implicated in C-type RNA viral release and an increased incidence of lymphoreticular malignancies (9). The relationship, however, among chronic immunostimulation, immunosuppression, and the development of lymphomas remains unclear. Moreover, the mechanism of immunosuppression is unknown. Virus particles, suppressor T cells, and macrophages have all been implicated (10–12). Recently Phillips (13) has demonstrated a suppressive substance released from thymic-dependent lymphocytes in the GVH reaction. This factor decreases the response of normal lymphocytes to mitogenic and allogeneic stimulation (1) and may be similar to previously reported soluble suppressors released during allogeneic reactions (14).

Previous work in this laboratory has demonstrated that alpha-fetoprotein (AFP) binds to murine T cells (15) and is a potent inhibitor of certain murine cellular reactions (16), including cytotoxic reactions (17), as well as the antibody response to T-dependent antigens (18). We have demonstrated, moreover, that it may function as a serum and lymphocyte culture supernatant suppressive substance in a group of patients with chronic active liver disease.[2] In addition, our recent work has shown that AFP is synthesized by tissue mononuclear cells (lymph node and spleen) in suppressed but not nonsuppressed patients with lymphoma (19). As previous authors have suggested a relationship between lymphoma and murine GVH (11), the present study was undertaken to examine the role of AFP in murine GVH reactions.

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[1] *Abbreviations used in this paper:* AFP, alpha-fetoprotein; GVH, graft vs. host; MAF, mouse amniotic fluid; MLC, mixed lymphocyte culture; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; SDS, sodium dodecyl sulfate; SI, stimulation indices.

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Materials and Methods

Animals. C3H/HEJ and C3H × C57BL/6F$_1$ (C3BF$_1$) age-matched (6-wk old) mice were used in all in vivo experiments. CBA/J and BALB/c mice were used for in vitro mixed lymphocyte culture (MLC) reactions. All mice were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Antisera. The method of purification of mouse AFP and transferrin, and the preparation of monospecific antisera to these proteins have been described (18). The antisera were shown to be monospecific by gel Ouchterlony immunodiffusion and immunoelectrophoresis. Fluoresceination of antisera to AFP was performed as previously reported (15).

In Vivo Allogeneic Reactions (GVH). C3BF$_1$ animals received 9×10^7 parental or syngeneic spleen cells (controls) on day 1 and were sacrificed at intervals of 3 days and 1, 4, and 8 wk. Before sacrifice, the animals were weighed and then exsanguinated under light anesthesia from the axillary artery. The spleens were dissected, the fat removed, and the organs were placed in preweighed cold sterile Petri dishes and weighed on a Mettler Balance (Mettler Instrument Corp., Princeton, N. J.). Simonsen spleen body weight assays were calculated and figures >1.3 were considered significant (20). Mesenteric and inguinal lymph nodes were dissected rapidly and both spleens and lymph nodes were transferred to sterile Petri dishes containing RPMI 1640 supplemented with penicillin (10,000 U), streptomycin (1,000 μg) and L-glutamine (200 mmol) (Grand Island Biological Co., Grand Island, N. Y.). The organs were individually forced through a 100 mesh sterile screen with gentle pressure. The suspensions were transferred to sterile test tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.), pooled, washed three times in media, resuspended in a convenient volume, and counted for use in in vitro experiments described below. The cells used to assess AFP and transferrin synthesis were washed two additional times in minimum essential media (MEM) lacking leucine (GIBCO), supplemented with penicillin and streptomycin. Viability, by trypan blue exclusion, was determined on all samples before and after the period of culture.

In Vitro Synthesis. The cells, diluted to 5×10^6 viable mononuclear cells/ml, were cultured in microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.) in 0.2 ml of MEM lacking leucine and supplemented with penicillin, streptomycin, glutamine, and 2½% heat-inactivated fetal calf serum (Microbiologic Associates, Bethesda, Md.). [^{14}C]leucine in a concentration of 0.4 μCi (sp act >500 mCi/mmol) (New England Nuclear, Boston, Mass.) was added at the initiation of culture or at predetermined intervals for pulse experiments and the cells were maintained at 37°C in a 5% CO_2 humidified atmosphere. At 6-h intervals the cells were pooled by pipetting and the supernates, after centrifugation, were stored at −70°C. The cell button was suspended in Nonidet P-40 (NP-40) (Shell Chemical Co., New York) at room temperature for 20 min and the cells were lysed by mechanical mixing. The suspension was centrifuged at 105,000 *g* for 30 min (Beckman Spinco L 65; Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and the liquid phase (cell sap) (21) removed by pipet. The button was resuspended in NP-40, allowed to remain for 2 h at room temperature and solubilized on a vortex mixer. All samples were dialyzed extensively against 0.14 M phosphate-buffered saline (PBS) and concentrated by Amicon filtration (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) before precipitation with specific antisera. AFP and transferrin synthesis in the various fractions were determined as described below. It is possible that the short incubation with NP-40 used to obtain cell sap extracted some membrane proteins. However, experiments in which cell sap and membranes were obtained by a repeated freeze-thaw technique did not differ significantly when compared with the method described above.

In Vitro Allogeneic Reactions (MLC). Two-way (bilateral) MLCs were performed according to a modification of the method of Peck and Bach (22). CBA/J and BALB/c spleen or lymph node cells, diluted to 2.5×10^6 viable cells/ml with MEM-lacking leucine (synthesis) or RPMI 1640 (thymidine incorporation), were placed together in the presence of 2½% fetal calf serum, previously shown to have minimal stimulatory activity. Control cultures contained each cell type alone. The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere for intervals of 1–96 h in the presence of 0.4 μCi of [^{14}C]leucine. In some experiments the cultures were not fed leucine but were pulsed 12 h before harvest with 1 μCi of [^{3}H]thymidine. The cells were harvested and prepared as described for GVH cells or were harvested on an automatic-harvesting device (Skaytron, Norway) and counted on a liquid scintillation counter. Stimulation indices (SI) were determined as the arithmetic mean

of triplicate samples of experimental divided by the sum of the mean of the controls.

In Vitro Mitogen Reactivity. $C3BF_1$ spleen or lymph node cells from experimental or control animals were reacted with purified phytohemagglutinin (PHA) (The Wellcome Research Laboratories, Beckenham, England) using three different concentrations of PHA in a microculture system previously described (16). The arithmetic mean of triplicate samples using the optimum PHA concentration was determined and the results are expressed as counts per minute ± standard error (SE) of the mean. Percent suppression was determined as $1 - [(\text{experimental})/(\text{control})] \times 100$.

Quantitation of AFP and Transferrin Synthesis. Samples from the GVH and MLC reactions were reacted with monospecific anti-AFP or antitransferrin antisera in the presence of mouse amniotic fluid (MAF), which contains significant concentrations of both of these proteins. The amount of MAF added was predetermined in quantitative precipitin reactions to yield a sufficient precipitate to work with and to be in antibody excess. The volumes were made equal with PBS and the samples incubated for 90 min at 37°C followed by incubation at 4°C for 18 h. The precipitates were washed three times in ice-cold PBS, resuspended in 0.1 M NaOH, and aliquots added to scintillation vials with aquasol and counted on a liquid scintillation counter. Synthesis is reported as counts per minute and represents the activity of 1×10^6 viable mononuclear cells.

Surface Fluorescence for AFP. Routine staining for AFP was performed as previously described (15). Briefly, 5×10^6 cells were reacted with 100 μl of specific antisera for 30 min at 4°C. The sample was washed three times with media, placed on a slide, and sealed. 100 cells were counted under white light and the percent demonstrating ring fluorescence under UV light recorded (E. Leitz, Inc., Rockleigh, N. J.). Previous blocking experiments (15) using purified mouse AFP and cold unlabeled anti-AFP antisera demonstrated the monospecificity of this reaction. Separate aliquots containing $5\text{-}10 \times 10^6$ mononuclear cells were reacted with 0.5 ml of MAF (containing 1 mg/ml of AFP) as previously reported (15). The samples were maintained at 37°C for 90 min, washed extensively with media, and reacted with antisera as reported above.

Radioimmunoassay. The double antibody technique was employed as described by Gleich et al. (23). Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis on 10% gels were done according to the method of Weber and Osborn (24), and the gels were frozen, sectioned into 2-mm slices, dissolved in 0.1% SDS, then placed in aquasol, and counted.

Results

A previous report (15) has shown that surface AFP is not detectable by immunofluorescence on normal adult murine spleen or lymph node cells and this was verified in the present study using both the C3H and C57BL strains. The results of experiments on cells from 8-wk GVH spleens are summarized in Table I and Fig. 1. There is striking staining on the surface of spleen cells for AFP. 33% (average) of mononuclear cells demonstrate surface AFP. This could be increased to almost half of the splenic cells if the suspension was preincubated for 90 min with MAF as an exogenous source of AFP. Normal spleen cells treated similarly with MAF show 18–22% of the cells with surface AFP. Lymph node cells from experimental GVH animals showed only low numbers of cells with surface AFP (0–6%). When lymph node cells are incubated with MAF, however, a slightly higher percentage (25–35%) bind AFP than do control non-GVH lymph node cells (24–26%). Controls consisted of $C3BF_1 \rightarrow C3BF_1$ or C3H and $C3BF_1$ alone, and no staining for AFP was seen in either spleen or lymph nodes.

The cells with surface AFP in the GVH spleens were both large, immature-appearing mononuclear cells and small, well-differentiated lymphocytes. We cannot state what type of spleen cells (T, B, or macrophage) has surface AFP in the GVH reaction. This is presently being investigated. Experiments with normal spleen and lymph node cells indicate that binding to the small lympho-

TABLE I
Percentage of Cells Having Surface AFP by Immunofluorescence in Splenic and Lymph Node Cells of Animals Undergoing an 8 wk GVH Reaction

	Cells with surface AFP*	Cells having bound AFP after incubation‡
	%	%
Control spleen	0	22–23
Experimental spleen	28–38	42–45
Control lymph node	0	24–26
Experimental lymph node	0–6	25–35

* Range for three separate experiments with 10 experimental and 5 control animals in each.

‡ Cells incubated in MAF (as a source of AFP) for 90 min at 37°C, washed, and stained at 4°C with a fluoresceinated monospecific anti-AFP antiserum. Ranges given for three separate experiments each including 10 experimental and 5 control animals.

cyte populations is restricted to a subset of T cells (15). In human lymphomas, however, T cells as well as unidentified large undifferentiated cells all bind AFP (20).

Since animals undergoing a GVH reaction have been reported to be immunosuppressed (1), we examined the mitogenic response of spleen and lymph node cells from control and experimental animals. These data are summarized in Table II. Spleen cells from 8-wk experimental animals stimulated by optimal concentrations of PHA reveal significant suppression (71%) compared to controls. Lymph nodes from experimental animals, however, were normal or showed slight augmentation compared to the PHA response of controls.

Since serum levels of AFP were similar in experimental (mean 500 ng/ml) and control animals (565 ng/ml), we questioned whether AFP might be synthesized directly by splenic cells during allogeneic reactions. The results of these experiments are presented in Tables III and IV. There is striking synthesis of AFP by splenic cells in both the GVH and MLC reactions as measured by [^{14}C]leucine incorporation, and SDS gel electrophoresis revealed ^{14}C incorporation only in the band with a mol wt of 76,000, which corresponds to the molecular weight of AFP by SDS gel analysis. In addition, supernates from MLC reactions in culture for 3 days were shown to have 1,000 ng/ml of AFP by radioimmunoassay, while control cultures had <75 ng/ml. No synthesis, however, is seen in controls (F_1 cells injected into F_1) or in either control cell type in the MLC. The data shown in Table IV were obtained using a two-way MLC reaction, but AFP synthesis, although less striking quantitatively, could be shown using the one-way MLC reaction in which the BALB/c cells were pretreated with mitomycin C before culture. Transferrin synthesis, reported to occur in small amounts by stimulated mononuclear cells (25), was used as a control. The amount of synthetic activity, however, was markedly less than that of AFP (GVH transferrin supernate,

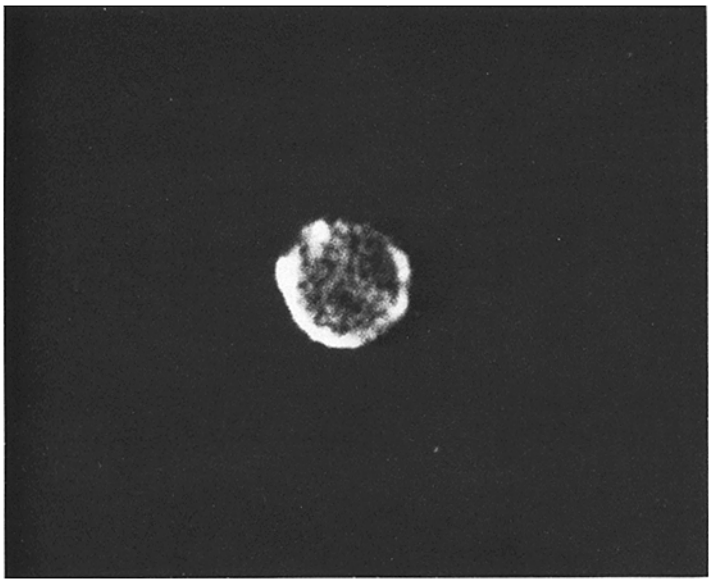

FIG. 1. A photomicrograph of a splenic lymphocyte from an 8 wk GVH reaction showing surface staining for AFP. × 400.

1,348–2,144; sap, 2,942–3,700; and membrane, 444–728: MLC supernate, 1,344–2,580; sap, 352–2,528; and membrane, 212–1,196 cpm). In addition, the curve of the magnitude of transferrin synthesis at various time points (not shown) was different than AFP, with the major synthesis of transferrin occurring earlier in the GVH and MLC reactions. These differences, as well as the demonstration of AFP synthesis both of [^{14}C]leucine incorporation and radioimmunoassay, show that AFP synthesis is marked (compared with transferrin) and specific in that it has different kinetics from transferrin.

Since immunosuppression has been reported (1) to vary during the course of a GVH reaction, we examined AFP synthesis over several time periods. These data are summarized in Table V and demonstrate that maximum AFP synthesis increases with the length of allogeneic stimulus for up to 60 days, despite similar indices of GVH reactivity (Simonsen assay, 1.45–1.49) at each point tested. Whether this relationship exists during the initial 7-day period when heightened immune reactivity has been noted (1) is under investigation. Preliminary data, however, show no surface AFP on 3-day GVH spleen cells and these cells demonstrate an increased response to PHA stimulation compared to control F_1 animals.

Another facet of these experiments is presented in Figs. 2 and 3. There is a second peak of AFP counts in the membrane fractions in both the in vivo and in vitro allogeneic reactions at a time when synthesis of AFP in the cell sap is decreasing. This pattern of reciprocal changes in membrane and sap AFP after the initial period of synthesis was observed in every experiment (eight GVH and four MLC reactions), although the absolute levels of the counts varied. In addition, the splenic MLC reaction revealed significant membrane AFP counts as summarized in Table IV with an average SI of 17 (range 15–19) while the

TABLE II
PHA Reactivity of Spleen and Lymph Node Mononuclear Cells from Animals Undergoing an 8 wk GVH Reaction

	Unstimulated	Stimulated	Suppression of experimental compared with control
	cpm	*cpm*	%
Control spleen	4,665 ± 32	150,649 ± 111	–
Experimental spleen	247 ± 14	44,749 ± 299	71
Control lymph node	253 ± 73	68,671	
Experimental lymph node	937 ± 337	87,007	0

cpm represents arithmetic mean of triplicate samples with presence of optimum PHA concentration (stimulated) or media alone (unstimulated).

TABLE III
Synthesis of AFP by Splenic Mononuclear Cells from an 8 wk GVH Reaction

GVH*	Experimental spleen of C3H → $C3BF_1$	Control Spleen of $C3BF_1$ → $C3BF_1$
	cpm‡	*cpm*‡
Supernate	4,480	162
Sap	15,144	186
Membrane	6,529	90

* Figures are cpm as AFP (mean of 10 animals) after 48 h of in vitro culture which was the time point showing maximum incorporation.
‡ Counts represent synthesis by 1×10^6 viable spleen cells.

lymph node MLC reaction showed negligible membrane AFP counts with a higher average SI of 50 (range 55–64).

The increased MLC reaction of lymph node cells compared to spleen and the cyclic pattern of AFP synthesis in splenic cells suggested that membrane AFP might act as a regulator of cell mitotic and/or synthetic activity with high membrane AFP acting as a negative feedback signal. The results of the experiments pertinent to this possibility are summarized in Fig. 4. In each instance, when membrane AFP increased, protein synthesis, as measured by total TCA precipitable counts, diminished in the next 12 h and alternatively, when membrane AFP was present in small amounts, total protein synthesis subsequently increased.

Discussion

We have demonstrated that splenic mononuclear cells obtained from 8-wk GVH mice have surface-bound AFP and that the spleens of these animals synthesize significant amounts of AFP. These data point out the important observation that nonhepatic tissues are capable of synthesizing AFP under appropriate stimulation. Although it seems likely that it is the splenic lymphoid cells that are synthesizing AFP, we have no direct proof of the cell type involved,

TABLE IV
Synthesis of AFP by Splenic and Lymph Node Mononuclear Cells from MLC Reactions

	Experimental	
	Splenic MLC reactions of CBA → BALB/c	Lymph node MLC reactions of CBA → BALB/c
	cpm	*cpm*
Supernate	6,264	4,128
Sap	8,364	408
Membrane	6,392	324

	Controls			
	CBA alone	BALB/c alone	CBA alone	BALB/c alone
	cpm	*cpm*	*cpm*	*cpm*
Supernate	214	260	316	208
Sap	24	30	68	164
Membrane	121	171	148	228

Figures are cpm as AFP(mean of four experiments) per 1×10^6 viable cells after 36 h of in vitro culture which was the time of initial maximum incorporation. The MLC was a two-way reaction. Spleen SI, 17 (range 15–19). Lymph node SI, 59 (range 55–64).

and macrophages or other as yet unidentified cells may be synthesizing AFP. Tumor (lymphoma) cells could not be identified in the spleens of these animals. The reports (26, 27) that embryonic antigens appear on the surface of cells undergoing blast transformation suggest that AFP may be produced by the cells undergoing activation in response to an allogeneic stimulus. In the MLC reaction the AFP-synthesizing cell would likely be the responder and not the mitomycin-treated stimulator cell, although, again, we have not ruled out macrophages.

The differences noted between the spleen and lymph node in the same GVH animals are of interest. Although the lymph node cells synthesized some AFP, there was no membrane-bound AFP by immunofluorescence and the levels of ^{14}C-labeled AFP in the membrane fractions of lymph node cells were significantly less than that in membranes of cells of splenic origin. This was not due to an absence of receptors for AFP on lymph node cells since both in this study and in our previous work (15) we have shown that incubation with AFP results in positive fluorescence in approximately 20% of the cells from both normal spleen and lymph node. It should be pointed out, however, that in the experiments where lymphoid cells are incubated with AFP, high concentrations (1 mg/ml) are employed. The differences, therefore, between the spleen and lymph node could be quantitative, and using lower concentrations of AFP may bring out differences in the binding properties between these cell types. This is of potential importance in view of the reports (28) of variations in the distribution of subpopulations of lymphocytes (amplifier vs. suppressor cells) in the lymph node

TABLE V
Maximum AFP Synthesis as a Function of the Duration of the Allogeneic Stimulus in GVH

Fraction*	7-Day GVH	30-Day GVH	60-Day GVH
	cpm	*cpm*	*cpm*
Sap	7,512	10,696	15,144
Membrane	1,144	4,756	6,529

* Values for cpm as AFP in sap and membrane fractions obtained after 48 h of in vitro culture which was the time of maximum incorporation.

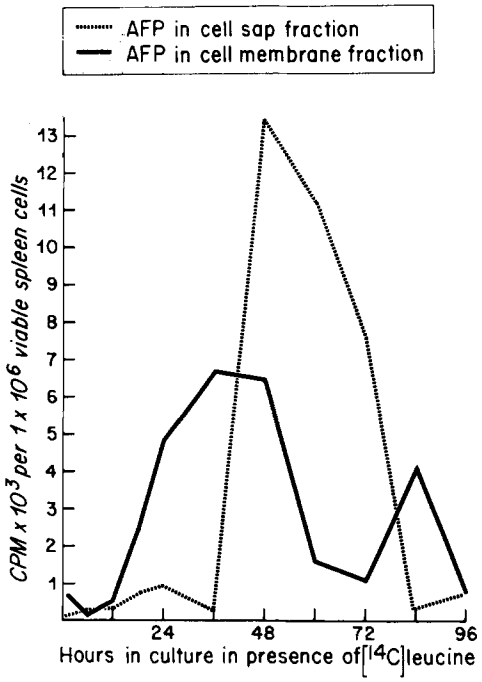

FIG. 2. Time study of AFP synthesis in spleen cells from a 60 day GVH reaction. The method of separating sap and membrane fractions and determining AFP as counts per minute is described in the text.

vs. the spleen. Perhaps these subpopulations have different affinities and/or numbers of receptors per cell for AFP and, therefore, differential susceptibility to suppression by this protein.

From reports in the literature (29, 30) it appears that lymph node cells are significantly more reactive than spleen cells in MLC reactions. The data summarized in Table IV support this and further suggest that these differences may be due to quantitative variations in the amount of membrane AFP. This possibility remains speculative, but preliminary data employing incubation with graded concentrations of AFP reveal there is significant difference in lymph node and spleen cells in their affinity for binding of AFP.

When spleen and lymph node cells from GVH animals were preincubated with exogenous AFP, greater numbers of cells showing surface AFP were found

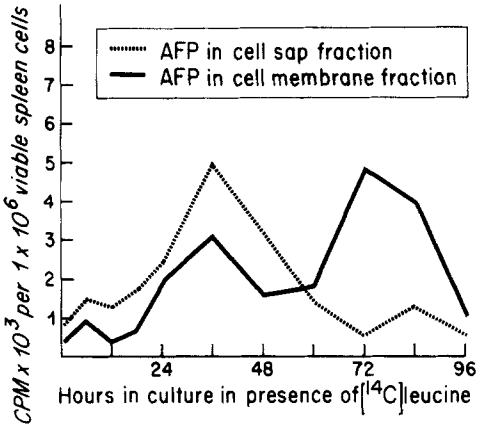

FIG. 3. Timed study of AFP synthesis in spleen cells from a bilateral MLC reaction. The method of separating sap and membrane fractions and determining AFP counts is described in the text.

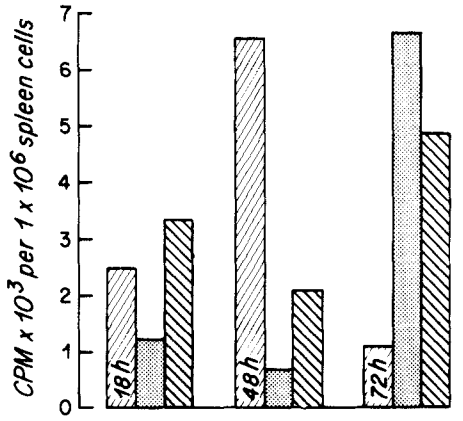

FIG. 4. GVH pulse experiments. (▨), counts per minute as AFP in membrane fraction of GVH spleen cells after 18, 48, or 72 h of incubation with [^{14}C]leucine added at initiation of culture. (▩), counts per minute as TCA-precipitable protein in cell sap fractions of cultures pulsed with [^{14}C]leucine after 18, 48, and 72 h of incubation and harvested 6 h later. (▧), counts per minute as TCA-precipitable protein in cell sap fractions of cultures pulsed with [^{14}C]leucine after 18, 48, and 72 h of incubation and harvested 12 h later.

compared with similarly treated cells derived from normal animals. This was especially marked with spleens from GVH animals which showed approximately twice the number of AFP-binding cells as control spleens. This could be due to a relative increase in GVH spleens of a special subset of T cells that binds AFP. It is also possible that activated lymphocytes or macrophages have an increased number and/or affinity of receptors for AFP. In previous experiments (15) we have shown that "activated" peritoneal macrophages obtained using thioglycollate (31) do not bind AFP, although this does not rule out binding by macrophages activated during a GVH reaction.

Our work, like many other previous studies (reviewed in reference 11), has

shown immunosuppression during a chronic GVH reaction. Whether AFP is involved in the immunosuppression, however, is at present speculative. There are, nonetheless, a number of lines of indirect evidence which suggest that this is a reasonable possibility. Firstly, we have previously shown that AFP is immunosuppressive for certain T-mediated reactions as well as antibody synthesis in vitro when present in microgram amounts (0.1–1 μg/ml) in the culture (17). Secondly, we have demonstrated that sera from pregnant mice and sera and supernates from lymphocyte cultures of patients with chronic hepatitis are immunosuppressive and that the immunosuppression is due to AFP (reference 32, and footnote 2). Thirdly, in a group of 10 human lymphoma patients we have recently reported (19) the presence of tissue and circulating lymphocytes with surface AFP by immunofluorescence and synthesis of AFP by splenic and lymph node tissues. These findings were restricted to those patients who were immunosuppressed and not to controls or nonsuppressed lymphoma patients, thus demonstrating a significant correlation between AFP synthesis and immune suppression in lymphomas. This is particularly pertinent in view of the reported high incidence of the development of lymphomas in animals with chronic allogeneic disease (13). Finally, more direct evidence that AFP is the immunosuppressive factor in supernates of mixed lymphocyte reactions has recently been obtained using affinity chromatography to specifically delete AFP. Removal of AFP abolishes the suppressive properties of MLC reaction supernates on the PHA and mixed lymphocyte response of normal cells.[3]

The binding and AFP synthesis by splenic and lymph node tissues in GVH reactions has implications in theories relating oncogenesis and immunity. In general, two theories have been proposed to explain the observed clinical and experimental association between immunity and malignancy. The theory of immune surveillance (33, 34) suggests that the immune system, particularly cell-mediated immunity, is a primary mechanism in the natural defense against the development of malignant tumors. An alternate hypothesis has been proposed more recently by several workers, particularly Prehn (35) and Melief et al. (11). Advocates of this theory refute many of the tenets upon which immune surveillance is based and stress the high incidence of malignancies of the lymphoid system in both immunosuppressed experimental animals and patients. Thus, there seems to be a special relationship btween immunosuppression and lymphoproliferative diseases rather than among all types of malignancy. It has been suggested that impaired regulation of the immune response, especially if resulting in immunostimulation may be of primary importance in the development of tumors rather than deficient surveillance. Hirsch et al. (10) have reported that antigenic stimuli activate latent endogenous oncogenic C-type viruses, suggesting that oncogenic viruses replicate more readily in transformed, stimulated, and/or deregulated cells. On the clinical side, the high incidence of lymphomas in immunosuppressed patients may be related to the antigenic stimulus of the allograft or the autoimmune disorder for which the

[3] Keller, R. H., and T. B. Tomasi. Alpha-fetoprotein: a soluble inhibitor of the immune response generated in mixed lymphocyte reactions. Manuscript in preparation.

patient is being treated with the immunosuppressive agent. In addition, the immunosuppressive agent itself, in an appropriate dosage, may selectively affect subpopulations of lymphocytes such as suppressor cells (36) and cause deregulation which in turn leads to proliferation and the appearance of oncornaviruses. Our data showing AFP synthesis in GVH reactions is consistent with either of these theories. The allogeneic stimulus resulting in blast transformation and synthesis of AFP could lead to defective tumor (surveillance) and/or antiviral immunity. No data is currently available on whether AFP activates latent viruses. It is not unreasonable, however, that AFP could promote viral proliferation since it inhibits both cell-mediated cytotoxicity (7) and antibody synthesis (18), both of which are known to be important in viral immunity. Further direct studies on the effect of AFP on viral infections are certainly indicated.

We believe an important aspect that is often neglected in discussions of the immune system and malignancy concerns the possibility of local or regional immune suppression in the environment of the tumor. For example, one of the points used to refute immune surveillance (37) is that animals and patients with early spontaneously arising neoplasms show little evidence of immune deficiency. It is possible, nonetheless, that the critical area to examine is not the systemic antibody or cell-mediated response of circulating lymphocytes but rather the immune response in the local environment. The production of small amounts of AFP by the tumor tissue itself or by lymphoid cells transformed in response to tumor-specific or viral antigens may be sufficient to inhibit the destruction of the first few malignant cells and allow them to gain sufficient numbers to escape the subsequent immune response. The possibility of regional production of AFP not reflected systemically is suggested by the finding of splenic AFP synthesis accompanied by immune suppression with normal or decreased serum levels of AFP and no evidence of immune suppression in lymph nodes. This shows regional suppression even within lymphoid organs.

The studies outlined in Figs. 2–4 demonstrate an inverse relationship between membrane-bound AFP and protein synthesis and suggest that AFP may have a more general role in the regulation of cell synthetic activities as a negative feedback signal. The important question of whether AFP plays any role in regulating normal adult lymphoid (or other) cells and their response to normally encountered antigens is presently under intensive study in our laboratory.

Summary

Surface-bound alpha-fetoprotein (AFP) was demonstrated by immunofluorescence on approximately 1/3 of splenic lymphocytes in chronic murine graft vs. host (GVH) reactions. Splenic lymphocytes were also shown to have a suppressed phytohemagglutinin (PHA) response compared to controls while lymph node cells from the same GVH animals revealed no surface AFP and had normal PHA responses. Splenic lymphocytes showed marked synthesis of AFP in the GVH and mixed lymphocyte culture (MLC) reactions by [^{14}C]leucine incorporation and by radioimmunoassay in MLC supernates. Lymph node cells, however, demonstrated less synthetic activity and ^{14}C counts in their membrane fractions were not markedly elevated.

The level of synthesis of AFP by spleen cells increased with the length of the allogeneic stimulus up to 8 wk. The pattern of AFP and total protein synthesis was shown to be cyclic. Pulse experiments with [^{14}C]leucine demonstrated that high membrane AFP was associated with a marked decrease in total protein synthesis by spleen cells in the next 12 h while low membrane AFP was associated with marked protein synthesis. This suggests that AFP could function as a negative feedback signal. These findings are discussed in relationship to the potential importance of allogeneic reactions and AFP in the interactions between the immune system and tumors.

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