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FETAL ANTIGENS IN NEOPLASIA[1]

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Most experimental oncologists would agree that their central focus in the past century has been a search for the elusive common denominator in cancer. Despite the fact that cancer is not a single disease, but encompasses a vast array of pathologies, oncologists refuse to believe that nature would select a multimechanistic method for permitting neoplasia. This is not to imply that many carcinogens, whether biological or chemical, do not serve to trigger neoplasia; the belief is simply that all serve to trigger the same operational defect in the cell or the host to permit invasive cell reproduction. Such thinking triggered Warburg's hypothesis regarding glycolysis and neoplastic growth. Similar thinking inspired the more recent membrane hypothesis offered by Sallach (1969), and the "oncogene" hypothesis propounded by Huebner and associates (1969).

There has been a proliferation of data in the last several years regarding another basis for a possible common denominator in cancer, that is, tumor-associated fetal substances. It has been known for a long time that analogies exist between fetal and malignant tissue: in patterns of transfer RNA, of enzymes (particularly those related to carbohydrate metabolism), and of different isoenzyme profiles (particularly lactic dehydrogenase and aldolaces). These biochemical differences, however, are quantitative in nature as well as often being associated with normal tissue undergoing regeneration or in conditions of stress.

The expression of fetal substances in non-malignant diseases has been known for years. A number of the fetal analogies which have been described are poorly understood. However, one well defined fetal moiety, fetal hemoglobin, has been well documented in several types of anemic conditions in man (Chernoff, 1953). While it is well established that the fetal

hemoglobin is a "phase specific" product during embryogenesis, it is also known to persist in adult life, associated with genetic disorders (Chernoff, 1953). Another phase specific fetal protein, alpha fetoprotein (αFP) has been detected in conjunction with viral hepatitis, at a time of hepatic regeneration (Geffroy et al., 1970; Sauger et al., 1970), as well as hepatomas (Tatarinov, 1964, Abelev, 1967). It is important to note that the phenotypic expression of these phase-specific proteins has not at present been linked to an autochthonous immunologic reactivity of the adult host.

The interest in the relationship between embryogenesis and oncology revolves around the elaboration in malignant cells of phenotypes characteristic of normal precursor cells at an earlier stage of development (embryonic or fetal). The nomenclature of early pathology abounds with descriptive correlations between neoplastic and undifferentiated tissues. Of more practical importance, however, is the renewed recognition of tumor associated fetal antigens, that is, antigens of tumor cells which are recognized in the host, but were present in early fetal life. The fact that these antigens are found on tumor cells has suggested their use as a diagnostic tool in cancer; the fact that they are found on fetal cells suggests a source of tumor antigen to be used in immunotherapy or immunoprophylaxis.

A recognized property of malignant transformed cells is their capability in many cases for these cells to produce unique substances which distinguish them from their normal cells of origin. Furthermore, these products can be functionally characterized, such as enzymes (Fishman et al., 1969), hormones (Lipsett, 1965), and distinct cell surface antigens. Along with the tumor specific antigens, it seems that there exists an additional class of antigens,

characteristic of embryonic progenitor cells. One class of tumor associated fetal antigens immunologically and biochemically characterized in man are the carcinoembryonic antigens whose presence and circulation is associated with malignancies of the digestive tract (Gold and Freeman, 1965).

An unsolved question regarding tumor associated fetal antigens is whether this "retrogenic expression" involves de novo synthesis of antigens on or in tumor cells, coded for by genes expressed or active in fetal precursors (Fig. 1). Another alternative is that one aspect of transformation is the unmasking of cryptic pre-existing antigenic sites. An example of this phenomenon was recently shown for trophoblastic cells by Currie and Bagshaw (1967). They demonstrated that isoantigens of trophoblastic cells are masked by sialomucins and unmasking and the expression of isoantigens could be accomplished by treatment with neuraminidase. In tumor cells this may or may not require activity on the transcriptional level. A further possibility that is very realistic when one considers the tumor and fetal analogies of quantitative changes in enzymes (particularly those associated with carbohydrate metabolism) is the incomplete synthesis or absence of cell surface carbohydrate side chains resulting in exposure of antigenic parts of the incomplete residues. This may also result from rearrangement within the macromolecule (Burger, 1971).

The fact that analogies exist between tumor and fetal cell surfaces has been shown by other than immunologic procedures. A characteristic of both fetal and cancer cells is the increased susceptibility to agglutination by plant lectins (Burger and Goldberg, 1967; Inbar and Sachs, 1969) and the appearance of specific glycolipids (Hakamori and Murakami, 1968; Burger, 1969).

It has been reported that these surface characteristics of fetal and tumor cells also appear transiently in some normal cells when they are in mitosis.

Detection of Tumor Associated Fetal Antigens in Allogeneic Systems:

One approach to immunological characterization of fetal antigens in experimental systems has been to raise antisera to tumor or fetal extract in an allogeneic system and then after suitable absorption, to test for immune precipitation. This procedure operationally would provide an efficient basis for detecting the "universality" of embryonic antigens in cancer tissues. Utilizing this method, Stonehill and Bendich (1970) reported on extracts obtained from 72 tumor specimens originating in 18 different mouse strains. The tumors were developed in 12 diverse tissues and were induced by either irradiation, various chemicals, or a variety of oncogenic viruses. It was demonstrated in these studies that rabbit antisera to mouse embryo extracts produced precipitin lines with extracts of all 72 cancers, and this reactivity could not be absorbed with most normal adult tissue.

Support for this finding was presented by Klavis et al. (1971), comparing antiserum, also generated in rabbits, against extracts of human fetal tissue, and testing against extracts of a variety of human carcinomas. Precipitin reactivity, which was unable to be absorbed by most normal adult tissue, was reported. An important point is that in both of these studies, reactivity of rabbit anti fetal serum was demonstrated for adult skin, by either immunofluorescence or precipitin reaction, and this reactivity could not be absorbed by adult normal tissue. Furthermore, Stonehill et al. (1972) subsequently demonstrated that the anti-fetal activity could be absorbed by adult skin extracts. This raises the possibility that in both studies

cross-reactive antibody could have been to collagen or connective tissue associated with skin. This is interesting, when one considers that the immunization of rabbits in both studies was performed by subcutaneous injection of fetal tissue extract and complete Freunds adjuvant. While both authors have argued that embryonic antigens represent different molecular species from the antigens of skin, i.e., the molecular weights of embryonic antigens within the range of 66,000 to 68,000, whereas the skin antigens have molecular weights of from 10,000 to 15,000, it is still possible that in part what is being measured is an antibody to an autoantigen. However, it has been brought to our attention recently that some embryo-specific tumor antigen activity was still present in anti fetal serum following absorption with skin extracts, using the radioimmune diffusion procedures (Stonehill, personal communication, 1973).

In experimental systems, the search for tumor associated antigens common to fetal cells has revolved around detecting antigens conferring transplantation immunity. The relevance of this is realized when one reviews the history of tumor immunology from the point of view of cross-immunity among tumors. Prehn and Main (1957) suggested the existence of limited degrees of cross-immunity among syngeneic tumors (methylcholanthrene-induced sarcomas in mice) and provoked hopes in tumor immunology. The degree of cross-immunity, however, could not be readily detected in later studies, so the concept was generally unheralded. The unique or "private" antigenicity of chemically induced tumors was then strongly emphasized by Klein et al. (1960), and the consensus then developed that tumors chemically induced in experimental systems hold no antigens in common, while common

antigens do exist among tumors induced by oncogenic viruses. However, more recently, exceptions to this generalization have been reported. In chemically induced strain-2 guinea pig hepatomas, Zbar et al. (1969) reported that 2 out of 5 tumors shared common antigens. A similar report was made by Holmes et al. (1971) for MCA-induced sarcomas in strain-2 guinea pigs. Usubuchi and co-workers (1972) have also suggested limited levels of cross-reactivity among various ascites tumors of non-inbred rats as well as mammary carcinomas and MCA-induced sarcomas of C3H/HE mice. Several other reports of cross-reactivity between some MCA-induced tumors exist in the literature (Old et al., 1968; Reiner and Southam, 1969; Hellstrom and Hellstrom, 1969). To add to this, some reports of limited cross-reactivity of cell surface antigens in melanoma and several other tumors in man have also been reported (Morton et al., 1970; Hellstrom et al., 1971). While these reports do not provide a basis for rejecting the generalization of distinct tumor specific transplantation antigens (TSTA) on chemically induced or spontaneous tumors of nonviral origin in animals, they do suggest caution in rejecting the possibility of some low level of crossreactivity and possibly stimulate the search for a level of existing common antigen among tumor cells. This seems to be where the fetal antigen concept opens the door for continued research and efforts along the lines of tumor associated fetal transplantation antigens. The data amassed on this subject in the last few years has been both impressive and provocative.

In 1962, Buttle and associates recon..med the earlier findings that immunization with fetal tissue induced transplantation immunity in cortisone treated rats, against the human sarcoma HS1. Similar results were obtained

in mice against chemically induced tumors (Buttle et al., 1964, 1967). It was pointed out in these studies that successful embryonic tissue immunization against tumors occurred primarily in nonsyngeneic systems; in other experiments where pure line BALB/c mice were used both as donors of embryonic tissue and as tumor hosts, the growth of tumors in mice injected with embryonic tissue did not differ significantly from that in the control, nor was there any prolonging of the survival time in the treated animals. The fact that methylcholanthrene tumors in pure bred mice do not respond to injection of embryonic tissue from the same strain suggested to these authors that the effect may be similar to that occurring in the homograft reaction, and may occur only when there are genetic differences between the host animal and the embryonic tissue used. An alternative interpretation lies in the fact that embryoma development occurred and neutralized the effect of immunization in this syngeneic system. This possibility is reasonable, based on the later finding that X-irradiated-viable fetal cells provide adequate protection against tumor transplantation in syngeneic systems.

Detection of Tumor Associated Fetal Antigens in Oncogenic DNA-virus Experimental Systems:

A critical dissection of the mechanism and techniques (Fig. 2) of fetal tissue transplantation immunity was provided by Coggin and associates (1970, 1971). Capitalizing on the early reports of Duff and Rapp (1970) which demonstrated that transformation of mouse and hamster cells by the oncogenic virus SV40 leads to the appearance on the cell membrane of a fetal antigen, Coggin and associates provided the following information. X-irradiation of fetal cells from time mated syngeneic females is a requisite to prevent

differentiation of the tissue in vivo and to prevent embryoma induction in vaccinees. It is essential to use fetus from primiparous females only, as fetus from multiparous females is less effective for immunizations and possibly is "coated" with IgG which masks immunogenicity. Multiple injection of fetus is required for effective immunization. Use of the correct age of fetus is important for maximal immunogenicity in order to obtain the phase specific antigens at their critical stage of development. These points have been demonstrated to be critical in the development of transplantation immunity in syngeneic systems.

The use of these techniques have provided the following information about fetal antigen expression and ability to provide protection against oncodna-virus-induced tumors of hamsters and mice (Coggin et al., 1970; 1971; Coggin and Anderson, 1972; Girardi et al., 1973). Males are rendered tumor resistant following immunization with syngeneic fetus. The effiency of fetal immunization is dependent on tumor challenge dose (Fig. 3). Females primed with fetus intraperitoneally develop antibody, but show no cell mediated immunity when tested in vivo or by in vitro procedure. Fetal antigens are phase specific, generally not being expressed in term fetus, but rather in early and mid-gestation fetus. Pregnant hamsters and mice (primiparous and multiparous) possess cytotoxic lymphoid cell populations which destroy tumor cells of many types but not "normal" cells or contact inhibited cells. Serum from multiparous hamsters can block the "tumor-specific" cytotoxicity of effector cells from tumor bearing animals and conversely, tumor bearing serum can abrogate the cytotoxic action of effector cells from pregnant animals in the SV40 system. Abrogation is

not so marked with pregnant serum that it is suspected that growth potentiating factors are present, as well as blocking factors. Fetal antigens seem to be autosoluble from tumor cells in vitro and may play a major role in producing blocking antibody which combines with circulating antigen and impairs cell-mediated immune reaction to the developing tumor.

An important consideration that must be made in evaluating the SV40 system is that Baranska et al. (1970) showed that the fetal-transformed cell surface antigen relationship goes back earlier in ontogenesis since antiserum against unfertilized mouse eggs cross-react with SV40 virus transformed mouse fibroblast (3T3) and not with other eggs or parent 3T3 cells.

Pearson and Freeman (1968) detected a common antigen of polyoma transformed hamster cells and hamster cells derived from 12-day-old fetus. R. C. Ting (1968) was unable to induce resistance to polyoma tumors in mice after immunization with unirradiated syngeneic mouse fetal cells. Later, however, using an isotopic antiglobulin technique and immunization with irradiated fetal cell preparations, C. C. Ting et al. (1972) demonstrated that anti fetal cell sera produced in male C3H mice reacted against cells from tumors induced by polyoma virus or SV40. Thus the overall results clearly indicate that cells transformed by oncodna viruses are capable of elaborating cell surface antigens which are common to fetal cells, and as pointed out by the studies of Ting et al. (1972), are clearly distinct from the tumor specific cell surface antigens. It is of interest to point out that a repressor-like mechanism in both SV40 and polyoma virus induced cell transformation has been recently described, which results in common and

distinct biochemical changes found in cell surface membrane components. Mora et al. (1969, 1971) described that after cell transformation with SV40 or polyoma virus there is a reduction in the content of the higher ganglioside homologs and in the activity of the transferase enzyme necessary for synthesis. This biochemical change is passed on as a hereditable property, together with the virus genome in stable transformed cell lines.

Detection of Tumor Associated Fetal Antigens in RNA-Tumor Virus Experimental Systems:

In 1971 and 1972, closely following the essential studies describing tumor associated fetal antigens in oncodna virus systems, several reports described similar cross-reactive antigen systems in RNA-tumor virus experimental models. Hanna et al. (1971) showed that the recovery of spleen cells infected with Rauscher leukemia virus (RLV) and grown in millipore diffusion chambers, the development of RLV-induced splenomegaly, and the cumulative mortality from a transplanted syngeneic, plasmocytoma were all modestly suppressed in BALB/c male mice previously primed with X-irradiated syngeneic fetal cells. As pointed out by these investigators, while their data showed a level of cross-reactivity between fetal and RLV-infected and transformed cells, the studies were not performed in such a way as to rule out the possibility that immunity elicited by fetal antigens is directed against common virion or non-virion transplantation antigens induced by the virus.

Ishimoto and Ito (1972), using sera collected from C57BL/6 mice immunized with RLV-infected cells, confirmed by indirect immunofluorescent test, cross-reacting antibodies against fetal cells and Rauscher leukemia cells. Absorption tests showed that this antigen was different from Gross (G) and

Friend-Moloney-Rauscher (FMR) antigens. They argued the possibility of the cross-reacting antigen, being a non-virion cell surface antigen induced by the virus (such as the E, ML and EL antigen systems described by Old and Boyse, 1965), was eliminated by the experimental model. A critical study with respect to this point was performed by Ting et al. (1972). In these studies an isotopic antibody technique demonstrated that antisera produced in male mice by inoculation of irradiated, syngeneic fetal tissue, reacted with cells from tumors induced by polyoma virus or SV40 virus. Furthermore, this activity could be removed by absorption of the serum with cells from tumors induced by murine leukemia viruses, including G virus (C58NT)D, RLV (RBL-5), as well as by chemical carcinogen (EL-4) and cells from mammary tumor and plasma-cell tumors. The activity could also be absorbed with fetal tissue. This work of C. C. Ting et al. (1972) again shows that fetal antigens may be expressed on a broad spectrum of tumor cells and more importantly, strongly suggests that these fetal antigens are distinct from tumor-specific antigens unique for the particular tumor or for tumors induced by a particular virus. Also, these studies provide an experimental basis for bridging the results with respect to fetal antigen expression in oncogenic DNA and RNA virus systems.

While the critical studies of tumor associated fetal antigens in murine leukemia virus experimental systems have demonstrated cross-reactivity of antibody, there is limited data demonstrating that an effective cell mediated component may be associated with this antigenic system. Tennant et al. (1971), using an <u>in vitro</u> cytotoxicity test, showed that spleen cells from mice challenged with syngeneic fetal tissue exhibit some weak reactivity against

syngeneic embryo cells and increased reactivity to cells infected with Moloney leukemia virus. Herberman et al. (1971) also demonstrated limited in vitro cell mediated bytotoxicity using effector cells from donors immunized with irradiated fetal cells for an allogeneic and syngeneic system using lymphomas as target cells.

Detection of Tumor Associated Fetal Antigens in Chemically Induced Tumor Models:

In 1967, Prehn suggested that tumor associated fetal antigens may be present in a chemically induced tumor model. The data were derived from a series of experiments in which some suppression of implants of syngeneic methylcholanthrene-induced sarcomas was produced in mice previously injected with fragments of syngeneic fetus. We now recognize that live fetal cell injections produce embryonic tumors (embryomas). Later, Brawn (1970) demonstrated that lymph node cells from multiparous BALB/c mice inhibited in vitro colony formation of several lines of primary syngeneic MCA-induced murine sarcomas. Lymph node cells from virgin females lacked this reactivity.

Baldwin et al. (1972) performed a comprehensive analysis of the occurrence and specificity of fetal antigens on transplantable MCA-induced sarcomas and dimethylaminobenzene (DAB)-induced hepatomas in rats. Membrane fluorescence and microcytoxicity tests showed that both tumors expressed fetal antigens at the cell surface which could be detected with serum and lymph node cells from multiparous rats. Again, in this study the fetal antigen was demonstrated to differ from the tumor specific cell surface antigens of the two tumors. It was also demonstrated in these studies that sensitization of animals with fetal cells did not confer significant tumor immunity.

A recent study by Grant et al. (1973), using inbred strain 2 guinea pigs and transplantable MCA-induced guinea pig sarcomas (MCA-25 and MCA-A), correlated delayed type hypersensitivity (DTH) as measured by skin testing to tumor cell membrane extracts, with MCA-25 tumor transplantation immunity. Animals were immunized with the same tumor, with MCA-A, with irradiated syngeneic fetal cells and also normal virgin females or multiparous animals were used. The results showed significant DTH in guinea pigs immunized with the specific tumor, fetal cells and in the retired breeders. In these various groups of animals the ability to develop delayed type hypersensitivity correlated with significant depression of tumor growth. The same group of investigators (Wells et al., 1973) have achieved significant tumor transplantation immunity in syngeneic fetal cell immunized or multiparous females using Fisher 344/N rats and a transplantable MCA-induced fibrosarcoma (MCA-R) and in C57BL/6 mice using a transplantable MCA-induced fibrosarcoma (MCA-10).

Effect of Tumor Cell Immunization on Fetal Development:

At present it could be stated that it has been adequately demonstrated in a broad spectrum of spontaneous, oncogenic virus transformed, chemical transformed systems that there was activation of synthesis of membrane patterns which the cell was producing and needed during embryonic development and that these membrane patterns can be detected immunologically. However, the contribution of host response to tumor associated fetal antigens with respect to tumor rejection reactions has not been fully documented. It has been demonstrated in some but not all systems that immunization with fetal cells confers varying degrees of protection to tumor challenge or tumor induction. However, it has not been clearly determined whether this host

response may in part be attributed to a nonspecific and even nonimmunologic reaction rather than true immunologic crossreactivity. One approach to determining in vivo whether there is immunologic crossreactivity, in the strict sense, would be to demonstrate that immunization with tumor could adversely affect embryoma development. Preliminary attempts to prevent the development of subcutaneous embryomas, induced with whole fetal homogenates, in intact hamsters immunized with SV40 tumors, have been unsuccessful, although in this system fetal cell immunization has a suppressive influence on tumor growth (Ambrose et al., 1971).

A quantitative in vivo assay (Fig. 4) to test for cross-reactivity between fetal and tumor cell antigens was carried out, using a modified colony-forming unit (CFU) technique (Salinas et al., 1972). Fetal liver was used as an immunogenic and test-cell population because this population of cells is primarily hematopoietic and upon adoptive transfer to irradiated recipients, proliferates to form clonal colonies in the spleen, furthermore, as described above, Buttle and associates (1964) have shown that in rat and mouse systems, immunization with fetal liver cells, in contrast to whole fetus, maximally suppressed growth of implanted tumors. A comparison was made of fetal colony forming units (FCFU), resulting from injection of various doses of 15-day old fetal liver cells into irradiated adult male recipients previously challenged with multiple injections of X-irradiated syngeneic fetal liver cells. The results demonstrated maximum suppression of FCFU (55%) with 5×10^5 cells. Using this system the effect of immunization with a BALB/c plasmocytoma on FCFU was performed and the results are shown in Table 1. Suppression of FCFU was found in PCT-immunized animals, and in

animals receiving an additional booster of irradiated fetal liver cells subcutaneously. This suggests that the embryoma growing subcutaneously as a result of injection of live fetal liver cells may be absorbing antibody during the interval between the last PCT immunization and the irradiation and adoptive transfer into the recipients. Immunization with a variety of tumors was also shown to have a depressive effect on FCFU in this system (Table 2).

Subsequent results by Parmiani and Della Porta (1973) demonstrated that immunization of females with an MCA-induced syngeneic tumor reduced the rate of pregnancy from 96% in control mice injected with normal adult tissues to 63% during the first pregnancy and from 92% to 50% during the second pregnancy. A stronger inhibition was found in the anti-fetal cell immunized females in which successful matings were as low as 39%. These observations demonstrated that the anti-tumor immunity was associated with an intrauterine destruction during the first part of embryonic development.

Is the Fetal Antigen a Forssman Antigen?

A very basic and important question is whether or not the crossreactive antigens in these systems may be Forssman antigen. Recently, the expression of Forssman antigen has been examined in relation to events in cellular transformation. Makita and Yousuke (1971) assayed the immunological reactivity of Forssman antigen on isolated membranes of BHK cells using a hemolysis-inhibition technique. The results confirmed their earlier observation that the Forssman antigen was demonstrable on polyoma virus-transformed BHK cells but not on the untransformed cells. Fogel and Sachs (1964) found similar results for BHK cells before and after transformation with Rous sarcoma virus. The presence of "cryptic" Forssman on untransformed BHK cells was demonstrated

by Burger (1971) after a brief treatment of the cells with proteases. The activity found with protease treatment was comparable to that observed for polyoma virus-transformed BHK cells. Somewhat in conflict with these reports, Hakamori and Kijimoto (1972), using antisera prepared against purified Forssman-hapten complexed with bovine serum albumin in a direct radioimmune assay, could not find a significant amount of Forssman reactivity on BHK cells or their transformants. In addition, they demonstrated a decrease in Forssman antigen with polyoma virus-transformation of hamster embryonic fibroblast (NIL) cells. Thus the occurrence or alteration in Forssman antigen has not been definitively established for transformation of cells.

Coggin et al. (1971) indicated that irradiated hamster and mouse fetal tissue contained antigens which evoked immunity to SV40-induced tumors in syngeneic hamsters. Forssman antigen has been shown for hamster embryo cells early in gestation (10 days prior to birth) and during the progressive development of the hamster (Noonan and Burger, 1971). These observations, along with those previously described for Forssman antigen, have generated the possibility that the reported immunity evoked against SV40 tumor cells could be the result of Forssman antigen on these cells. This possibility was examined by the immunization of hamsters with irradiated guinea pig kidney cells, sheep erythrocytes and SV40 tumor cells (three immunizations at weekly intervals). Some of the animals from each test group were bled prior to challenge with SV40 tumor cells. After challenge, the animals were monitored for tumor appearance. The collected sera were tested for antibody titer against Forssman antigen. The sera were assayed also from tumor-bearer

animals, from animals at different stages in tumor development, from hamsters hyperimmunized with 10 day hamster fetus, and from primiparous and multiparous female hamsters at the 10th day of gestation.

Immunization with either guinea pig kidney cells, or sheep erythrocytes failed to prevent or retard SV40 tumor development, although these animals demonstrated a high titer for antibody against Forssman antigen in a hemolysis assay. A very weak antibody titer (1:2) relative to that produced with sheep erythrocytes (1:1024) or guinea pig kidney cells (1:512) was found for hyperimmune 10 day fetus antisera, hyperimmune SV40 tumor cell antisera, and for the other sera tested for this antibody. Thus although fetal and transformed cells have been shown to possess Forssman antigen on their cellular surfaces, there was no protective cellular response to Forssman containing sheep erythrocytes or guinea pig kidney cells. These results demonstrate that the fetal antigen(s) envoking immunity against SV40 tumor cell challenge, are most likely distinct from the Forssman antigen.

CONCLUSION

Two challenges have been made to immunologists with respect to the cancer problem: 1) What can immunology offer to the prospective cancer patient from the point of detection and diagnosis; and 2) what can immunology offer the patient with an established, disseminated tumor. Now we can ask in this context, whether or not tumor associated fetal antigens afford a means of meeting these challenges.

With respect to the first challenge, what is clear from the knowledge at hand is that cancers of common pathology in man may have cross-reactive

antigens. The extent of this is not clearly defined. However, from the data summarized in this review it is plausible that one common antigenic system may be embryonic or fetal in nature. To be sure, tumors of endodermal origin express carcinoembryonic antigens.

Operationally, immunologists could, and indeed have, proceeded without much more information to develop tests for detecting cancer, using various anti-fetal or anti-carcinoembryonic reagents. As can be seen, this approach is problematic at best, since no one has identified and characterized these antigens to a desirable degree. Specifically, we cannot at the present time answer the questions, whether there is a variety of fetal antigens; for example, one for each cell type, or only a few common antigens. This question will be resolved once the identification and characterization of species-, organ-, and phase-specific embryonic antigens common to tumor cells has been accomplished. Because of the complexity of working with the human model, we predict that this will be achieved best using experimental model systems.

With respect to the second challenge, the potential role of fetal antigens in immunotherapy, we would have to point out that in the experimental systems, the protection offered by multiple fetal cell inoculations is frequently no better than that obtained by nonspecific immune stimulation. A similar statement could also be made for use of TSTA in tumor bearing hosts. Again, our lack of knowledge is a major limitation. Specifically, we cannot at the present time determine if in all cases these tumor associated fetal antigens are autoantigenic. We do not know if these antigens will elicit humoral, cell-mediated immune responses, or both.

If membrane-bound, will they generally serve as antigenic targets for effector cell mediated cytotoxicity. Also, it is possible that in the autochthonous host these tumor associated fetal antigens could contribute to tumor progression by development of blocking complexes. The answers to these questions are being derived from the studies in experimental model systems.

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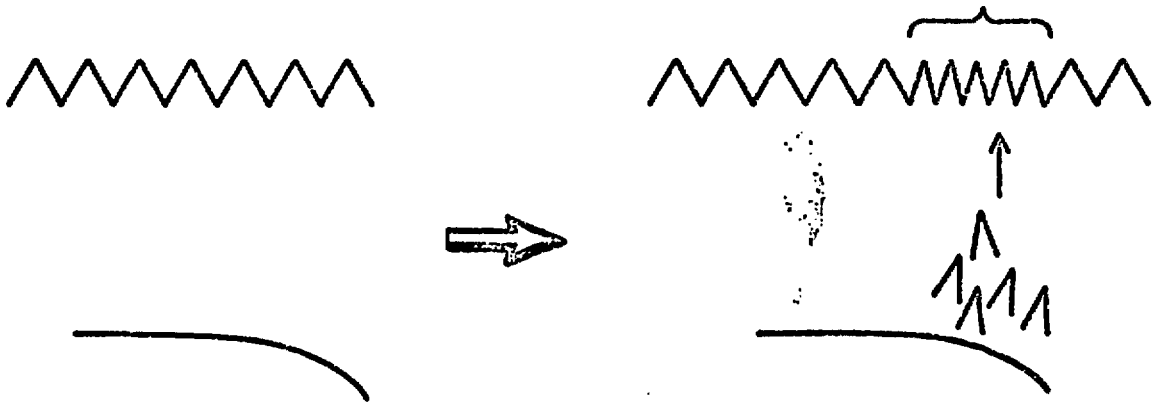
Figure 1. Possible alternative representations of "retrogenic expression" of tumor associated fetal antigens.

Figure 2. Techniques and procedure demonstrating the development of transplantation immunity in syngeneic fetal systems.

Figure 3. Effect of cell challenge level on detection of fetal induced transplantation immunity against SV-40 tumor cells.

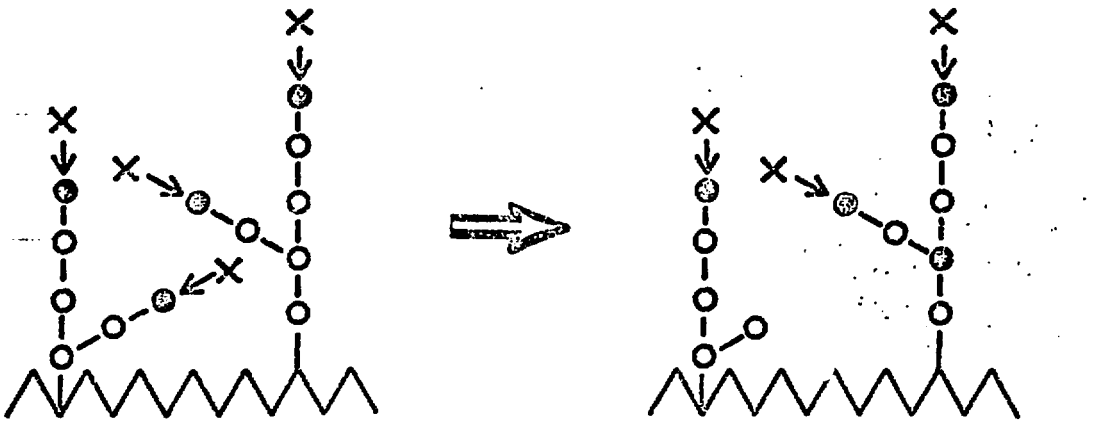
Figure 4. Quantitative <u>in vivo</u> assay for specific fetal and tumor antigen immunization (schematic).

Figure 1



De novo SYNTHESIS

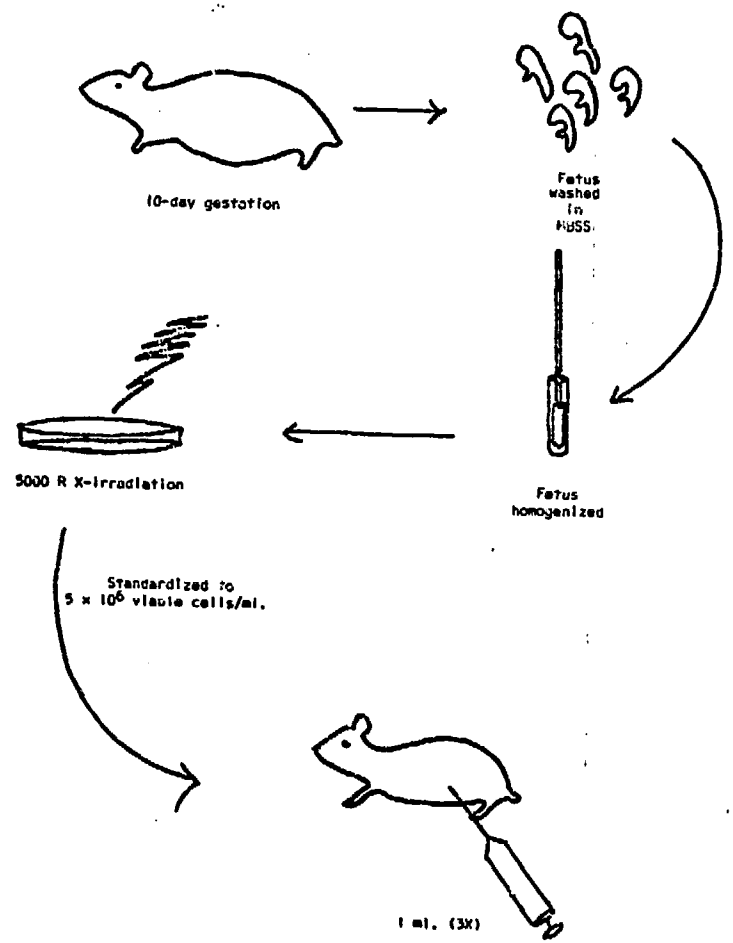
UNMASKING



ADULT FETAL OR TUMOR

INCOMPLETION OR ALTERATION

PREPARATION OF FETAL CELL VACCINE



EFFECT OF CELL CHALLENGE LEVEL ON DETECTION OF
FETAL INDUCED TRANSPLANTATION IMMUNITY AGAINST SV40 TUMOR CELLS

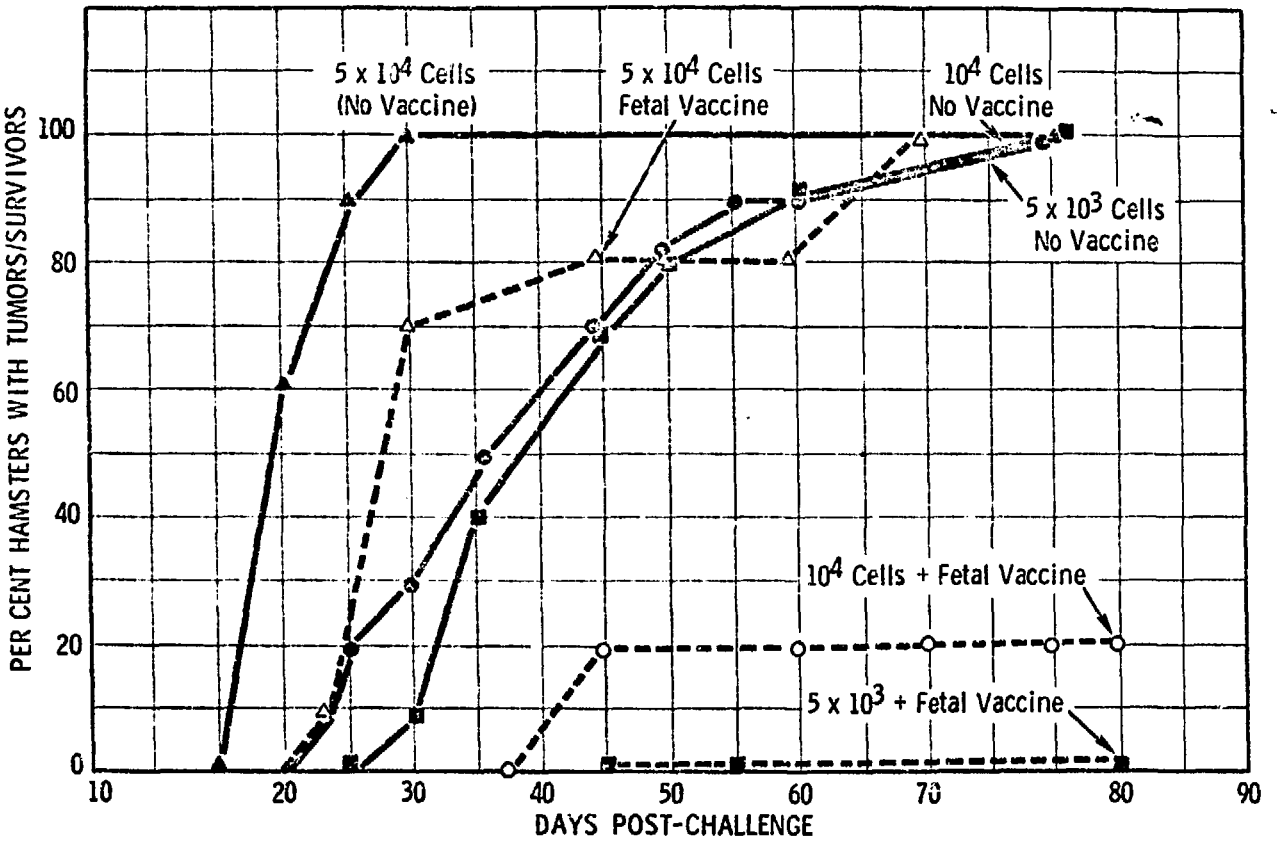


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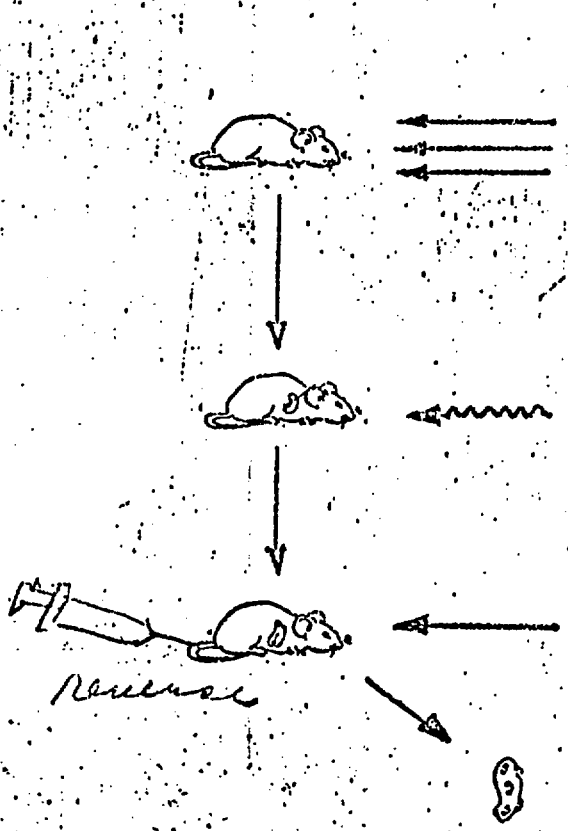
Figure 1

QUANTITATIVE IN VIVO ASSAY FOR SPECIFICITY OF FETAL AND TUMOR ANTIGEN IMMUNIZATION.

(A schematic representation)



Immunization SCHEDULE

N times injected i.p. with a known number of X-ray tumor or fetal syngenic cells.

At specific intervals after hyperimmunization whole body lethal irradiation (750 R).

I.V. injection of syngenic fetal liver cells.

Spleen colony counted at day 8 (FCFU.).