STUDIES OF ORGAN-ASSOCIATED ANTIGENS AND OTHER MARKERS IN HUMAN TUMORS WHICH MAY BE USEFUL FOR THE DIAGNOSIS OF MALIGNANT DISEASES

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The main rationale for these projects is to help identify test procedures that could pick out individuals in a population who might have been exposed to carcinogenic agents and would be the ones who would actually be coming down with malignant disease. This is a generally important problem, to be able to screen general populations or high-risk populations and to identify the relatively small numbers of individuals who harbor occult malignancies.

The particular objectives in this area have been to focus on immunologic markers that might be useful to diagnose certain types of human malignancies, particularly in this type of screening approach, and the focus for the contracts that are being supported under this program has been primarily leukemia and breast cancer, but these projects also have possibilities for other types of cancer.

There are some particular goals to keep in mind that are needed to be successful with tests of this type. I might note at the outset that accurate detection of occult malignancies is a very difficult task to accomplish, although it is obviously quite important.

One of the first criteria that one would be concerned with would be to have a test with a high degree of specificity. Particularly, it would be desirable to have a test that would be able to not only detect the presence of cancer but would be able to get some indications for the type of cancer or where it would be arising. This is one of the reasons to put some emphasis on markers that would have some tissue or organ association.

The other side of the coin is to have tests or markers which would have a high level of sensitivity. There are many tests in which the markers are positive with cancers when they are present at a fairly advanced stage, when there is a lot of tumor present. It is considerably more difficult for a test to be positive in patients with small amounts of tumor, when it is early, localized, and at a point that is treatable. That is clearly the stage that one would like to have sensitive tests, in order to be able to begin or alter therapy and thereby affect the course of disease.

Finally, if one is going to develop assays that might be valuable for screening of populations, even quite high-risk populations, this would involve testing of quite large numbers of individuals. Therefore, it would be necessary to have an asssay procedure which would be practical for large numbers of specimens and would, also, be quite reproducible.

There has been quite a large effort in this direction. The National Cancer Institute specifically has many grants and contracts that have been designed to develop immunodiagnostic or other diagnostic markers for cancer. The two projects that are being supported under this program essentially are just a small subsegment of this type of approach, but they are ones that were thought at the time of initiation of the program to be ones that were promising for these goals.

The first particular project that we are concerned with is at the University of Minnesota and has focused on leukemias and lymphomas. The investigators involved with this are Drs. Kersey and Tucker Lebien.

The particular approach that they started out with was to prepare heterologous antisera, primarily in rabbits, that would react against antigens that would be associated with various types of human leukemias, either in a quite specific way for the leukemias, or that would see differentiation antigens, that would not be entirely leukemia specific. The latter type might still be quite useful as a detection system since in the normal development of the hematopoietic cells these markers might only be present in a very small proportion of cells or in early stage of development. Thus, in this situation, one would be focusing more on quantitative differences rather than on real qualitative differences.

The approach which has been adopted during the past year or so has been to shift away from the more classical approach of just injecting cellular or subcellular materials into rabbits or other species, and to go to an approach which in general seems to be much more promising for specificity and, also, for developing large amounts of the same reagents. This involves the production of monoclonal antibodies in mice that might be able to see the same or perhaps an additional series of antigens.

It may be worthwhile to first briefly describe the methodology of monoclonal antibodies. Cells or subcellular materials are injected into mice and, at a time thought to be optimal for getting an immune response, spleen cells are taken from the mice. These lymphoid cells are co-cultured with an established myeloma cell line, preferably a myeloma cell line which itself does not make antibodies or immunoglobulins. The transformed cells are fused with the B cells that are in the spleens of the immunized animals; this fusion occurs quite readily in the presence of polyethylene glycol.

After fusion, the cells are cultured for a period of time in so-called "HAT" medium which is a combination of cytotoxic agents to which the parental myeloma line is quite sensitive. Thus, the unfused cells would be eliminated and only the fused products that have resistance to these materials would be able to survive. Supernatants from surviving cells in various wells are screened for the reactivity that one is trying to measure. This is the most critical part of this methodology and one has to be clever enough to have a good screen. The type of screens that the group in Minnesota has used has involved the use of paired cell lines; on the one hand, the leukemia line to look for positive antibody reactivity against leukemia associated antigens and on the other hand, an autolygous B cell line transformed with Epstein-Barr virus as the negative part of the screen. Thus, they are looking for antibodies that would react with the leukemic line and not with the B cell line from the same individual. They have been able to derive several clones from these fusions that have looked quite promising for antibodies that could be useful to detect leukemia-associated or differentiation antigens. One of these monoclonal antibodies was raised against a T cell acute lymphocytic leukemia line, HSB. This particular monoclonal antibody has not been nearly as selective as one would like, at least in the peripheral blood since it reacted with a large majority of T cells among peripheral blood mononuclear cells. However, it has reacted with a series of acute leukemias and leukemic cell lines but not with other types of acute leukemias.

There have also been some monoclonal antibodies that have been raised to another acute lymphocytic leukemia cell line, NALM-6. One monoclonal antibody to NALM-6 reacted to both the ALL line and the autolygous B cell line, and it appears to be detecting some B cell associated antigens. In the peripheral blood it seems to be reacting with more or all normal B cells, but in the bone marrow it seems to be much more selective, reacting with very few of the cells. For the screen against leukemias it has been quite effective in picking up all of the B type chronic lymphocytic leukemias and a series of acute lymphocytic leukemias.

The third monoclonal antibody that they have raised seems to be the most interesting one. This reacts against an ALL-associated antigen which seems to be very similar, if not identical to one which they had previously identified by heterologous antiserum. The specificity for this antigen seems to be a differentiation antigen associated with pre-B cells. Very small numbers of normal B cells in the bone marrow react with this, but almost no cells in the normal peripheral blood react. Yet, the antiserum reacts with a considerable portion of acute lymphocytic leukemias, mainly those which are classified as the most common type of ALL, the so-called "non-T, non-B" ALL and also reacts with so-called "pre-B ALL" and also with the cells from chronic myelogenous leukemias in blast crisis. This last group is particularly of interest because those types of leukemias have been asociated with some of the carcinogenic effects seen with cytotoxic therapies in cancer patients.

Although the first two antibodies that I described have not been nearly as selective as would have been hoped, one of the promising aspects to the overall study is that among 40 different individual leukemias that have been screened, there was essentially no overlapping between leukemias positive with one of these antibodies compared to the others. Thus, by using a panel of these three reagents it was possible to pick up almost all of the leukemias whereas each antibody detected only a subset. This may be the type of approach which will be taken in this area, to combine several markers since one particular reagent may not be sufficiently sensitive or specific.

The other project which is being supported in this area is at Emory University with Dr. R. Chawla as the principal investigator. The focus has been on a marker called EDC1.

EDC1 is a protein which has been found in the urine of cancer patients. It is a relatively low molecular weight glycoprotein. It has been possible to identify EDC1 in the urine of the majority of patients with metastatic breast cancer, and a variety of other types of solid malignancies.

This is not a tumor-specific protein but is actually a degradation product of a normal serum protein, inter-alpha-trypsin inhibitor, that is present in everyone's serum that has a much higher molecular weight (170,000). Under normal circumstances the serum protein is not degraded to produce detectable levels of EDC1. Therefore, the focus in most of the studies of Dr. Chawla has been to screen the urine for the lower molecular weight compound. The problem with this approach has been that the antibodies that he has available have cross-reacted immunologically between the serum protein and EDC1.

Therefore, he was faced with the task of finding a method to more specifically detect EDC1. To a certain extent, screening in the urine provided a biologic distinction because the large molecular weight protein under normal circumstances would not be filtered in the glomeruli, whereas the lower molecular weight one would. The problem with this approach is that with any impairment of glomerular function, there could be some leakage into the urine of the higher molecular weight material.

Dr. Chawla has corrected for that to a large extent by making a ratio of the amount of immunologically active material detected to the creatinine that was excreted into the urine. However, this was not entirely selective. He has been able to develop a radioimmunoassay which is much more sensitive than the early ones. However, it detects both proteins, and there have been some false positives in tests on urine which are probably related to the subtle impairments in renal function which can occur in some tumor-bearing individuals.

Dr. Chawla has been trying other ways to discriminate between the two proteins. One possibility was two-dimensional crossed immunoclectropheresis, since the charge, as well as the size of these molecules, was different. However, although the initial studies looked very promising, with clear separation of the two proteins, there have been some normal individuals who have had an unexpected degree of heterogeneity, with a peak of an intermediate molecule between the one and the other. This has caused some confusion in the assay.

The more recent thing that Dr. Chawla has come up with has been to precipitate the parent molecule with sulfa-salicylic acid. This seems to be quite effective in precipitating the normal serum protein without affecting the lower molecular weight compound. This seems to be a very good basis for an assay for distinction.

The objective of Dr. Chawla's project, once he works out this technical problem, is to screen a large number of sera and urine which he is currently collecting from patients who are being evaluated for the initial diagnosis for breast cancer. There are specimens from patients with breast cancer and also sera from women who turn out to have only benign breast disease.

At this time, with metastatic breast cancer patients, the large majority have levels of EDC1 that are above the range that is seen in either normal individuals or in patients with a variety of benign diseases, including benign breast lesions. Elevations also have been seen in a variety of other types of malignancies, including acute myelogenous leukemia. The hope is that with the radioimmunoassay, which is much more sensitive than the assay used to generate the current data, and some kind of selection procedure, there may be a specific and sensitive procedure suitable for the ultimate objective of this project.

Dr. Kraybill, NCI: We received a call the other day from an authority in the South Dakota State Department of Health. He has come across some records of 1400 tests that were run with a high indication of association of another biochemical marker, hyaluronidase, that might indicate tumorigenicity in people. Have you heard anything about this particular marker?

Dr. Herberman, NCI: I am not aware of detailed documentation that hyaluronidase is useful. There have been a series of enzymes that have been put forward as possible discriminants. In fact, my experience has been that investigators do a study by comparing advanced cancer with normals and see some very interesting differences.

Unfortunately, you can get the same kind of differences very frequently with the erythrocyte sedimentation rate. The bigger difficulty is to be able to get adequate discrimination between cancer, particularly at a localized stage, and benign diseases, especially of the same organ system as the cancer.

As part of the NCI's program we have a serum bank in which specimens are sent to investigators that have assays that they think are promising for making these kind of discriminations. Over about the five-year period that this has been available, there have been about 200 or so assays that have been screened, including a number of enzymatic assays of the type you have mentioned. Unfortunately, only a handful of them has had significant levels of discrimination when tested with the various groups of patients and controls that I mentioned.

Dr. O'Conor, NCI: I would like to raise a general and somewhat provocative question. I wonder whether you would comment on the potential of the three-dimensional system that Norman Anderson has at Argonne. I think you are familiar with it. Would you comment about where that might fit into this whole problem?

Dr. Herberman, NCI: I think this is a very impressive type of methodology. It has the ability, as I understand it, to look at a very wide cross section of metabolic products and not only immunologic but, also, biochemical types of markers. It might well pull out something which would be quite interesting.

With the serum bank that has been available there have been some things in the sera that have looked to be quite discriminating. For example, Dr. Phyllis Brown in Rhode Island has been looking at liquid chromatographic separation of nucleosides that are in sera. There are several nucleosides that she has been able to identify that seem to have considerably different patterns in cancer patients, including some early stage cancer patients, compared to some benign diseases and normals. Certainly the Anderson type of approach should be able to pick up this as well as a variety of other things. I guess one of the concerns that I have with that aproach is that you may be really flooded with information. There will be so much information gathered that it may be difficult to actually sort out the relatively small number of interesting or important differences from the background of so many other nondiscriminating materials. The other thing that I would have to add is that, although this technology is quite intriguing as an approach, I am not aware that it has been validated for this kind of application. We will really just have to wait and see how well it will do. Unfortunately, among the many potential tests screened thus far, there has been a low yield of tests that have held up under scrutiny. A very small proportion of tests can discriminate between known early cancers and known benign diseases and even fewer have the levels of sensitivity and specificity to screen even a high-risk population. For screening you need extraordinarily high levels of

sensitivity and specificity and I am not convinced that there are any tests that are adequate for screening for the types of cancers that we are concerned with in the United States.

Dr. Morris, EPA: As I understand, in the past with breast cancer we had HCG and CEA and one other test which I cannot remember now that was used in a battery approach. Do you feel that we are far enough along to consider some type of battery even at this point or do you feel that is premature?

Dr. Herberman, NCI: I think the battery approach probably will be the way to go. The problem is to decide what to put into the battery. My view is that each of the tests in the battery has to, on its own right, be reasonably powerful.

Some of the evaluations that have been done have included some things in the batteries that are marginal, and if you put together a few marginal tests, you seem to end up with marginal data in the aggregate with their problems adding up as well as their strengths.

In fact, the combination of tests for breast cancer that you are mentioning is an example of the problems in this area. HCG is a marginal marker for breast cancer.

Dr. Morris, EPA: You are saying that for high-risk groups it is far enough along, determining, you know, malignant disease versus benign disease. Do we have enough information or should we start considering this. I was thinking about my ACB population for example that are potentially at risk. Do we have enough information even in the present battery, admitting we may wind up with some benign in the process? How significant would it be, even at this point to approach that with some of these selected populations, not broadly but in select cases?

Dr. Herberman, NCI: I think an example that one can think about specifically in this regard is CEA (carcinoembryonic antigen). If one looks at cancers, carcinomas for example, versus a normal healthy population, it actually performs rather well, in that with colon cancer, breast cancer, lung cancer, at least 50 percent or more of patients have elevated levels of CEA as compared to less than 5 percent of the normal population. That sounds pretty good, but there is, also, a range of benign diseases with elevated levels. The experience has been that when CEA is applied for screening, most of the elevations are due to benign diseases since they are much more prevalent in the population than the cancers. For every cancer that may be picked up, there may be 50 or 100 benign diseases. In addition, the problem with markers of this type is that they tend to increase in their levels or even in their detection rate with more advanced disease. Thus, most of the cancers that were picked up were advanced and untreatable.

The one test which has really shown promise and has been applied at a large-scale level for screening has been alpha fetoprotein. The Chinese have utilized this test for screening in a high-risk area and have shown reasonably well that they could use this in field conditions. They could identify a significant number, at least, of people with lever cancer, including some surgically resectable liver cancers. However, the yield of really treatable cases was very low for the number of people that they had to screen, which has been in the neighborhood of one-half million. At most 10 or 20 treatable liver cancers were picked up.

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